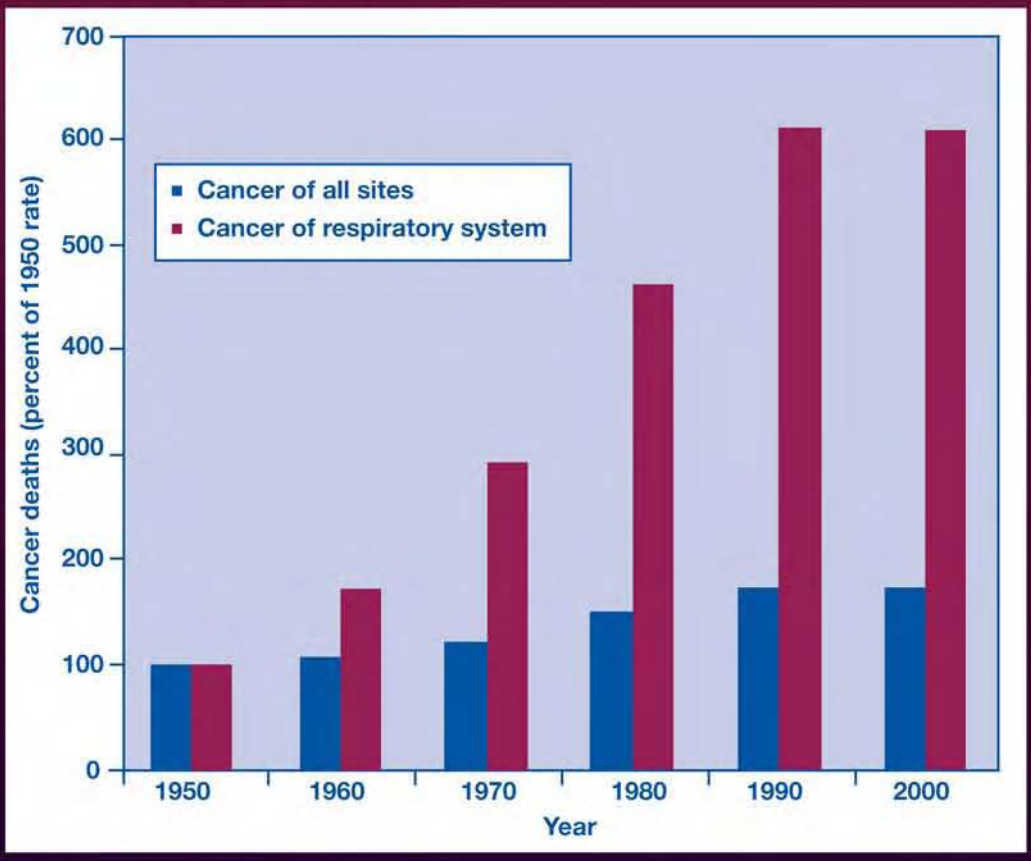
ENVIRONMENTAL TOXICOLOGY

Second Edition

Biological and Health Effects of Pollutants



Ming-Ho Yu

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On the Cover

The cancer death rates in the U.S. increased steadily from 1950 to the middle of the 1990s, when the increase began to slow down. Important differences are found between cancer death rate from all causes and the cancer death rate from respiratory system failure. For example, between 1950 and 1990, the increase in cancer death rate from all causes was about 70%, while it was over 500% for respiratory system cancer death rate. The marked increase stresses the important role played by exposure to increasing levels of air pollution (p. 26).

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Preface

This book is written with the objective of providing fundamental knowledge concerning the biological and health effects of environmental pollutants on living systems. The book emphasizes the chemical and biological characteristics of major pollutants found in our environment and their impacts on the health of living organisms, including not only humans and animals but also plants.

The volume consists of seventeen chapters. The first chapter, *Introduction*, introduces the reader to the theme of the book. The chapter begins with a definition of environmental toxicology and discusses the relationship between human activities and their impacts on the environment. This is followed by a brief history of environmental pollution and related laws in the U.S. The chapter ends with discussion of the importance of environmental toxicology as a field of study. Chapter 2, *Environmental Change and Health*, presents an overview of our changing environment, with statistics of the major causes of deaths in the U.S. from 1950 to 2000. A possible link between our changing environment and the changing pattern of human diseases is discussed. Included in the discussion are such diseases as cancer, birth defects, reproductive damages, respiratory diseases, and heavy-metal-induced diseases. Emphasis is also placed on the relationship between developing economies and growing pollution-related health problems in several countries. Chapter 3, *Occurrence of Toxicants*, identifies ways in which the occurrence of environmental toxicants may be recognized. This is followed by a brief review of major environmental pollution episodes or disasters that occurred in recent decades. Chapter 4, *Toxic Action of Pollutants*, discusses general ways in which environmental toxicants may cause deleterious effects on living organisms. The chapter includes processes involved in toxicant uptake, transport, storage, metabolism, action, and wherever applicable, excretion, highlighting several ways in which toxicants cause damage to plants, animals, and humans. Chapter 5, *Factors Affecting Xenobiotic Action*, discusses several factors that influence the toxicity of xenobiotics (environmental toxicants). Included in the discussion are physical and chemical characteristics of toxicants, environmental factors, biological factors, and nutritional factors.

The metabolism of environmental chemicals – biotransformation – is discussed in Chapter 6. The chapter introduces Phases I and II reactions and stresses the importance of biotransformation for living systems and the consequences of the process. Main topics covered in this chapter include detoxification of xenobiotics, possible production of free radicals by biotransformation, and the action of cellular antioxidant defense systems, including endogenous antioxidants and free radical scavenging enzymes. In Chapter 7,

Defense Responses to Toxicants, several major defense mechanisms available to help the animal or human body to cope with environmental toxicants are discussed. Emphasis is placed on such mechanisms found in respiratory tract, gastrointestinal tract, liver, kidneys, and membranes. A brief discussion of defense mechanisms manifested by some plant species is also included.

Chapter 8, *Air Pollution – Inorganic Gases*, deals with the four gaseous air pollutants included in the "Criteria Air Pollutants" designated by U.S. Environmental Protection Agency (EPA), i.e., sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3), and carbon monoxide (CO). The sources, characteristics, and health and biological effects are discussed. Particulate matter, also one of the Criteria Air Pollutants, is presented in Chapter 9. The sources, characteristics, and toxic effects of particulates are reviewed. A more in-depth discussion is presented for silica (SiO_2), beryllium (Be), and asbestos. Although fluoride is not designated by the EPA as one of the Criteria Air Pollutants, it is nevertheless an important atmospheric pollutant. Moreover, in contrast to other air pollutants discussed in Chapter 8, fluoride can exist in gaseous and particulate forms, and it is a waterborne pollutant as well, afflicting tens of millions of people in several countries in the world. The problem is worsening in several countries experiencing a growing use of coal for energy. The importance of environmental fluoride is, therefore, examined in Chapter 10. Volatile organic compounds (VOC), another group of pollutants belonging to the Criteria Air Pollutants, are discussed in Chapter 11. The properties and health effects of alkanes, alkenes, and aromatic hydrocarbons are reviewed in this chapter. Of the aromatic hydrocarbons, benzene, toluene, and the xylenes are discussed. Additionally, the sources, properties, health effects, and metabolism of polycyclic aromatic hydrocarbons (PAHs) are presented.

Chapter 12, *Soil and Water Pollution – Environmental Metals and Metalloids*, considers in some depth the sources, characteristics, health, and biological effects of several metals and a metalloid found in soil and water. Included in the discussion are lead (Pb), cadmium (Cd), mercury (Hg), nickel (Ni), and arsenic (As). The discussion includes a brief review of the incidents of itai-itai disease and Minamata disease. Chapter 13, *Pesticides and Related Materials*, presents the three groups of synthetic organic pesticides: chlorinated hydrocarbons, organophosphates, and carbamates. The chapter also discusses the toxic effects of several related organic compounds, such as PCBs, PBBs, and dioxins. Current concerns about the disruption of mammalian endocrine systems by these toxicants are also addressed.

Because of the growing attention towards widespread endocrine disrupting chemicals found in the environment, a new Chapter 14, *Endocrine Disruption*, has been included to enhance understanding of the issue. The chapter begins with a brief introduction, stressing the concerns shared by many scientists, followed by a review of hormonal function. These are followed by characteristics of endocrine disrupters, proposed mechanisms of their actions, and examples of endocrine disruption observed in various countries.

Chapter 15, *Mutagenic Pollutants*, deals with the types of mutation, common mutagens found in our environment, and their actions. The induction of mutation by ultraviolet and ionizing radiations, and chemical mutagens is reviewed. Of the chemical mutagens, examples are given to show alkylation, intercalation, and the interaction of several metals with DNA, leading to mutation. These discussions are then followed by Chapter 16, *Environmental Cancer*, which examines various environmental toxicants related to cancers. The chapter begins by stressing the importance of cancer to public health and discusses the known and proposed causes of cancer, including the stages involved in carcinogenesis. Emphasis is placed on various types of chemical agents, such as free radicals, vinyl chloride, alkylating agents, and polycyclic aromatic hydrocarbons, that are capable of interacting with DNA in initiating carcinogenesis.

The last chapter, Chapter 17, presents a brief introduction to ecological risk assessment, a relatively new but growing area of environmental science. The framework for ecological risk assessment, the importance of emergence of risk assessment as a regulatory paradigm, and the widespread use of ecological impacts to influence regulatory and policy decisions are discussed.

This volume is written primarily as an introductory textbook for upper level undergraduate and beginning graduate students majoring in environmental science, environmental toxicology, environmental health, public health, and other related fields. To assist the students in their study, review questions are included at the end of each chapter. A glossary is also provided as Appendix 1.

Much of the material contained in this volume is based on the lecture notes that I used in teaching environmental health and toxicology and related courses for 27 years at Western Washington University. I was encouraged by favorable responses expressed by my students. Many of them are now working in the area of environmental toxicology and related areas. It is hoped that students as well as professionals interested in enhancing their knowledge of the health and biological impacts of pollutants on living organisms will find this volume a useful text or source book. I welcome any suggestions from instructors and readers so that their suggestions can be incorporated into a possible future edition.

Acknowledgments

I wish to express my hearty appreciation to Dr. D.K. Salunkhe and Dr. G.W. Miller, both Professors Emeritus at Utah State University, for having me as a graduate student to work in their laboratories. Their guidance has contributed much to the teaching and research career I followed after my graduation. Several instructors kindly reviewed the first edition of my book and made valuable suggestions. Wherever possible, I have incorporated their suggestions into this edition. I am indebted to my wife Ervena for her support and assistance throughout my preparation of this volume. In addition, my appreciation also goes to the members of the editorial office at CRC Press for their patience and assistance.

Ming-Ho Yu

Author

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Chapter 1

Introduction

1.1 STUDY OF ENVIRONMENTAL TOXICOLOGY

Environmental toxicology deals with the effects of environmental toxicants on health and the environment. Environmental toxicants are agents released into the general environment that can cause adverse effects on the health of living organisms, including humans, animals, and plants. The study of environmental toxicology stems from the recognition that (a) human survival depends on the well-being of other species and on the availability of clean air, water, and food; and (b) anthropogenic chemicals as well as naturally occurring chemicals can cause detrimental effects on living organisms and ecological processes. The study of environmental toxicology is thus concerned with how environmental toxicants, through their interaction with humans, animals, and plants, influence the health and welfare of these organisms.

1.2 WORLDWIDE DEVELOPMENT IN RECENT DECADES

Enormous industrial and economic development has taken place since World War II. An example of such development is related to the chemicals industry, leading to the manufacture of a large number of chemical products. A worldwide use of many of these products, particularly fertilizers (Figure 1.1), insecticides, and herbicides then followed. This, together with the development of new high-yield grains, led to dramatic increases in world food production. Many food-deficient countries, including China and India, became capable of producing sufficient quantities of grain food to meet their domestic needs. Several traditionally food-importing countries even became food exporters. This remarkable achievement is widely known as the *Green Revolution*. Dr. Norman Borlaug, recognized by many as the *Father of the Green Revolution*, received a Nobel Prize in 1972 for his contribution to world grain production.

The dramatic increase in food production, coupled with technological advancement and rise in industrial output, led to an overall global economic expansion. Significant increases in gross national product (GNP) were witnessed in many countries. These developments, concomitant with improved medicine and medical science and technology, helped improve general public health. For example, life expectancy and infant mortality, which are measures often used to gauge the overall health of a population, have improved. Over the past 50 years, overall mortality has declined substantially among Americans of all ages. In 2001, life expectancy at birth for the total population reached a

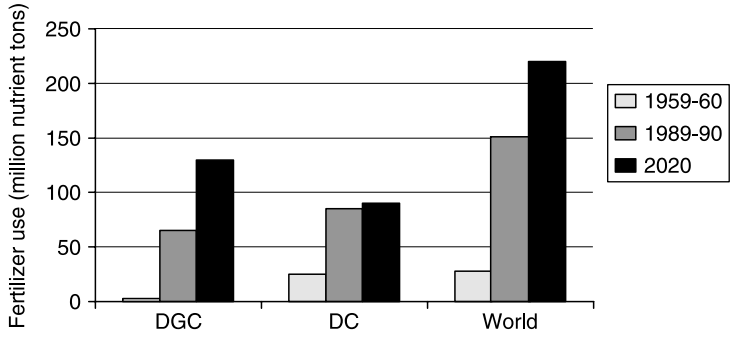

Figure 1.1 Growth in fertilizer use, 1960 to 1990, and prediction to 2020. DC = developed countries; DGC = developing countries.

record high of 77.2 years, based on preliminary data, up from 75.4 years in 1990.[1] During the 20th century, life expectancy at birth increased from 48 to 74 years for males and from 51 to 79 years for females. Similarly, in 2000 the infant mortality rate declined to a record low of 6.9 infant deaths per 1000 live births. Between 1950 and 2000, the infant mortality rate declined by about 75%.

Worldwide, life expectancy has risen to an average of 65 years and death rates have declined, especially among young children. In the wealthiest developed countries, average life expectancy rose from about 67 years in 1950 to 77 years in 2000. In the less-developed countries, life expectancy jumped from 40 to 64 years (Figure 1.2). In Brazil, for example, between 1940 and 1980 mortality declined from 18 per 1000 to 6 per 1000 persons, and life expectancy at birth increased by 20 years during the same period.[2]

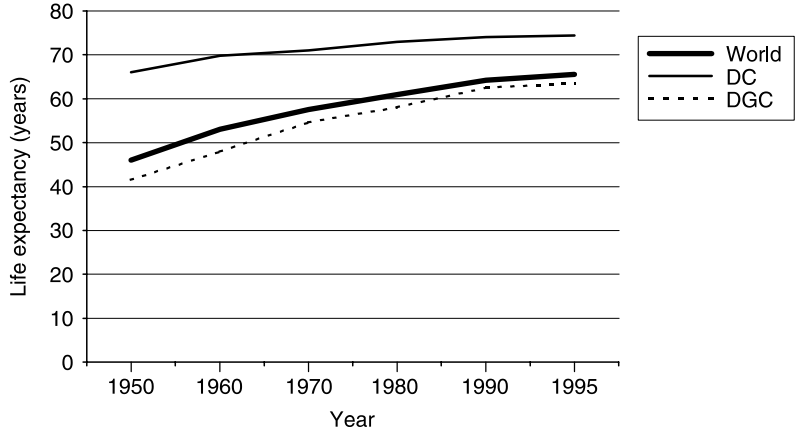

Figure 1.2 Trends in life expectancy, 1950 to 1995. DC = developed countries; DGC = developing countries.

1.3 ENVIRONMENTAL POLLUTION AND LAW

While many of the world's people were enjoying the benefits of technological and economic expansion and higher living standards, many scientists and the public became aware that this extraordinary development was not without cost. Indeed, the impact of global environmental changes that have accompanied development in various areas has become a growing concern. One such concern is the impact on human health. As early as in the 1950s and 1960s, many urban dwellers and residents living in the vicinity of industrial plants began to recognize undesirable changes occurring in the environment, particularly a general deterioration of the quality of air and water. A great deal of both field and laboratory research was conducted, with the results revealing the seriousness of environmental pollution problems. Subsequently, it was widely recognized that there was an urgent need to curb further deterioration of the environment and protect human health against the adverse effects of environmental pollution.

Recognition of the need led to the establishment in many countries of new national policies on the environment, particularly in the more developed countries. In the U.S., the National Environmental Policy Act (NEPA) was signed into law on January 1, 1970. Concomitantly, the Council on Environmental Quality was established with the responsibility for studying the condition of the nation's environment on a regular basis. In the same year, the Environmental Protection Agency (EPA) was established to be in charge of the environmental programs within the U.S. Increased awareness of the effects of environmental pollution on living systems, particularly on humans, has precipitated legislation and regulation around the world. In the U.S., the first Clean Air Act was written in 1970 and it has been amended three times since, in 1974, 1977, and 1990. This legislation was actually a compilation of amendments to an earlier one, but was tighter and required the establishment of ambient air quality standards with a margin of safety such that the most sensitive people would suffer no adverse health effects.

In 1971, the EPA identified six pollutants as requiring a national ambient air quality standard. These pollutants were:

- particulate matter
- sulfur dioxide (SO_2)
- carbon monoxide (CO)
- nitrogen dioxide (NO_2)
- photochemical oxidants
- hydrocarbons

They were known to influence human morbidity and mortality, and to have adverse effects on visibility, materials, vegetation, and other factors related to public welfare. The EPA also specified for the first time that the federal government would determine the best available technologies to be used in achieving performance standards for industrial plants, automobiles, and other

sources of air pollution. The 1990 amendment of the act calls for a "program of research, testing, and development of methods for sampling, measurement, monitoring, analysis, and modeling of air pollutants."

Several Water Pollution Control Acts have been passed in the U.S. since 1948; however, the landmark legislation did not come until the 1972 Federal Water Pollution Control Act was passed. The establishment of the act was in response to the deep public concern about the environment being voiced in the late 1960s. This act requires the determination of effluent limitations, i.e., limits on the materials that can be discharged into waters from factories, sewage treatment plants, and other point sources of pollution. In addition to water monitoring, reduction and removal of aquatic pollutants were also included in the act. Subsequently, the Safe Drinking Water Act was enacted in the U.S. in 1974. This was the first act intended to standardize the purity of water throughout the U.S.

The Toxic Substance Control Act (TOSCA) (PL 94-469), passed in 1977, called for regulation of "chemical substances and mixtures which present an unreasonable risk of injury to health or the environment." The act is unique because it gave the EPA the power to insist that new chemicals be considered guilty until proved innocent. The law recognizes two broad categories of chemicals, old and new. The EPA was assigned to assess the risks associated with the old chemicals, while the chemicals industry was to be responsible for evaluating the health and environmental effects of the new ones. The result of such legislation has been an intense effort to develop methods for evaluating toxicity, predicting environmental impacts, monitoring effects, and mitigating disasters.

1.4 IMPORTANCE OF ENVIRONMENTAL TOXICOLOGY

The field of environmental toxicology is consequently drawn in two synchronous directions: regulation and research. Regulation ensures standardized testing that is fast and economical, with results that may be applied in a general fashion. This has resulted in an emphasis on simplified scenarios, such as the traditional mortality test that uses only one test species and one test compound. Toxicological research, however, increasingly reveals the importance of complex interactions between individual organisms, species, physiological processes, environmental factors, and multiple anthropogenic chemical substances.

A comprehensive approach is emerging in the form of "risk assessment," as defined by the EPA.[3] This approach incorporates scientifically derived information with social and economic concerns, to appraise the potential consequences of particular human-induced stressors on the environment. Risk assessments often culminate in the development of a model for predicting long-term effects of a toxicant on environmental factors. However, such models may not be transferable from one site to another as no two sites have identical characteristics. The challenge of environmental toxicology now is to identify

the common principles that might allow extrapolation and prediction of the effects of toxicants on the environment.

Environmental toxicology diverges from traditional pharmacology or toxicology. The traditional methods for testing rely on the use of standard test organisms and laboratory methods to indicate relative toxicity of the various compounds in question. Instead, ecotoxicology addresses a more elaborate set of concerns. How are pollutants transformed after their release into the environment? How are organisms exposed, and how do physiological alterations impact on population dynamics and community structures? What indirect impacts occur to unexposed organisms when their prey, predators, or competitors are affected? How do the impacts of multiple compounds differ from those of a single one? Such questions are beyond the domain of one-organism, one-compound laboratory tests. Ultimately, ecotoxicological impacts will be elucidated through a combination of long-term field observations and use of assays and models.

The tools of the environmental toxicologist include biological assays, such as for studying individual growth, mortality, reproduction, metabolic rate, enzyme induction, etc. Field observations, including tissue concentrations of toxicants, species number and density, and population dynamics, are crucial. Field experiments, such as the containment of test organisms at contaminated sites and environmental simulations (microcosms and mesocosms), aid in the construction and testing of theories. Finally, data are often integrated into theoretical models – mathematical predictions of bioaccumulation or of species survival, for example. The goal of this test is to provide essential knowledge concerning the biological responses of individual organisms to pollutants. Direct toxicity to the organism is the fundamental route by which other effects, such as the influence of altered prey populations on predators, are mediated. With a thorough understanding of the nature of the major pollutants found in the environment and their biological impacts, the environmental toxicologist will hold the basic tools for research integrating other aspects of the field.

Environmental toxicology is a multidisciplinary science that encompasses several diverse areas of study, such as biology, chemistry (organic, analytical, and biochemistry), anatomy, genetics, physiology, microbiology, ecology, soil, water, and atmospheric sciences, epidemiology, statistics, and law (Figure 1.3). Compared with many other fields of study, environmental toxicology is a relatively young branch of science. However, its importance as an area of study has been widely recognized. Indeed, it is one of the most rapidly growing fields of study. This is obvious based on the large number of papers and books published in the past two to three decades that relate to environmental toxicology. Similarly, courses of environmental toxicology and related subject areas are being taught at a growing number of colleges and universities. Such a trend is not limited to the U.S. and Canada alone. Rather, it is widespread globally.

The founding of the Society of Environmental Toxicology and Chemistry (SETAC) is another example. This international society was launched in 1980, and 85 people participated in the first meeting held in Washington, D.C. The society's membership has grown markedly during the past two decades,

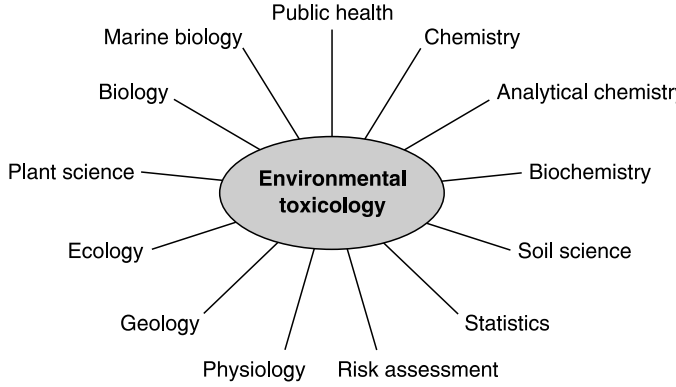

Figure 1.3 Interrelationships between environmental toxicology and various scientific disciplines.

reaching approximately 5000 individuals from 70 countries in 2004. SETAC's official journal, *Environmental Toxicology and Chemistry*, was launched in 1982 and has since become widely recognized as one of the most influential publications in the field of environmental science. The society holds an annual meeting in November in different cities in the U.S., where 2000 to 3000 individuals participate in presentation or discussion of new findings.

Clearly, a large number of scientists in the U.S. and various other countries are pursuing careers directly or indirectly related to environmental toxicology. The importance of their contributions to the enhancement of environmental quality and human welfare has become increasingly recognized.

1.5 TOXICITY TESTING – A BRIEF REVIEW

1.5.1 Introduction

This section presents a brief introduction to toxicity testing. Several terms used in environmental toxicology are also included. *Toxicity* is the property or properties of a material that produces a harmful effect upon a biological system, and the agent that produces such a biological effect is termed as a *toxicant*. The majority of the toxic chemicals discussed in this volume are of man-made or anthropogenic origin. It is true that some of the materials that are produced naturally by biological systems are extremely potent, e.g., the fungal aflatoxins and venom; however, these materials are usually produced only in small amounts. In contrast, anthropogenically produced materials can amount to millions of kilograms per year.[4]

Toxicants enter the environment by a variety of routes from many different sources. Toxicants introduced into the environment may come from two basic sources: *point* sources and *nonpoint* sources. Discharges from point sources include sewage discharges, waste streams from industrial sources, hazardous-waste disposal sites, and accidental spills. Point discharges are generally easy to

identify in terms of the types, rates of release, and total amounts of materials released. In contrast, nonpoint discharges are much more difficult to characterize. These include materials released from atmospheric emissions, agricultural runoff, contaminated soils and aquatic sediments, and urban runoff from such sources as parking lots and residential areas. In most situations, discharges from nonpoint sources are composed of mixtures of complex materials. Therefore, the amounts of toxicants released are difficult to assess, and the rates and timing of discharges are difficult to predict.

Many classes of chemicals can exhibit toxicity. One of the most commonly studied and discussed is the pesticide. *Pesticide* can refer to any substance that exhibits toxicity to an undesirable organism, but its toxicity often constitutes a broad spectrum. Industrial chemicals also are a major concern because of the large amounts transported and utilized. Metals, such as cadmium from mining and manufacturing processes, and as contaminants in oil, are also released to the environment from various sources. Crude oil and the petroleum products derived from it are significant sources of environmental toxicity because of their common usage and persistence in the environment. Importantly, many of these materials, particularly metal salts and petroleum, can occur in usually uncontaminated environments. However, the fact that a toxicant is present does not necessarily mean it will have a toxicological effect. Any chemical substance can exhibit harmful effects if the amounts introduced into an organism are sufficiently high. Indeed, the *dose*, or actual amount of material that enters an organism, can determine the biological ramifications. At low doses, it is possible that no apparent harmful effects will be observed. In many toxicity evaluations, however, an increase in the growth of the exposed organism may be observed when the dose of the material is very low, while at high doses mortality may occur. The widely used *dose–response relationship* refers to the relationship between dose and biological effect. In some instances, no effects will be observed until a certain threshold concentration is reached.

1.5.2 The Dose–Response Relationship

In environmental toxicology, environmental concentration is often used as a substitute for knowing the actual amount, or dose, of a chemical entering an organism.[4] However, in determining the relationship between dose and response, it is necessary to distinguish between the dose or environmental concentration and the amount of the material that reaches the target tissue. In some cases, the concentration of a compound may be high in the environmental medium, particularly if it is water soluble, but if the chemical is not absorbed and thus does not reach the target organ, no effect will be observed.[5]

The fundamental basis of the quantitative relationship between exposure to an agent and the incidence of an adverse response is the *dose–response assessment*. Dose–response usually refers to the relationship between measurable physical, chemical, or biological responses following exposure to a certain quantity of a chemical. Because, in most experiments, it is almost impossible to determine the dose of a chemical actually reaching the target tissue, the

environmental concentration or the amount of the chemical administered is used as the dose. The reaction to the dose, or the response that is elicited, can be quantitatively determined, either by the magnitude of the response or by the time taken for a specific response to be observed.

A graph that depicts the response of an organism, population, or biological community to a range of concentrations of a xenobiotic is the *dose–response curve*. Criteria such as the extent of DNA damage, behavioral changes, fatality, enzyme inhibition, and other responses can be described using this relationship.

In a study to find out the response, manifested by mortality, of an organism to an administered chemical, the distribution of mortality vs. dose (or concentration) of the chemical is drawn so that the cumulative mortality is plotted against each of the concentrations used. At each dose, the total number of organisms that have died is plotted. Table 1.1 presents data for a typical response to the concentration or dose of a particular agent. At each concentration the percentage or actual number of organisms responding or the magnitude of effects is plotted (Figure 1.4). The distribution of results resembles a sigmoid curve.

The presentation in Figure 1.4 is an example of the dose–response curve. Data are plotted as continuous and a sigmoid curve is the usual result. Two parameters of this curve are used to describe it: (a) the concentration or dose that results in 50% of the measured effect, and (b) the slope of the linear part of the curve that passes through the midpoint. Both parameters are necessary to describe accurately the relationship between the concentration of the chemical and the effect. The mid point on the curve is commonly referred to as a LD_{50}, LC_{50}, EC_{50}, or IC_{50}. The definitions of these are:

- LD_{50} – LD stands for *lethal dose*; LD_{50} is the dose that causes fatality in 50% of a sample group of an organism.
- LC_{50} – LC stands for *lethal concentration*; LC_{50} is the concentration that causes fatality in 50% of a sample group of an organism.
- EC_{50} – EC stands for *effective concentration*; EC_{50} is the concentration that has an effect on 50% of the test group of an organism, estimated by graphical or computational means. EC_{50} is often used for reporting effects other than fatality.

Table 1.1 Toxicity Test Data for a Chemical Agent

	Dose (mg/kg)								
	0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Cumulative toxicity (%)	0	2.5	8.0	20.0	75.0	93.0	98.0	100.0	100.0
Combined deaths at each concentration (%)	0.0	2.5	5.5	12.0	55.0	18.0	5.0	2.0	0.0

Note: The toxicity data are given as a percentage of the total organisms in a particular treatment group. For example, if 8 out of 100 organisms died or exhibited other end-points at a concentration of 2.5 mg/kg, the percentage responding will be 8%.

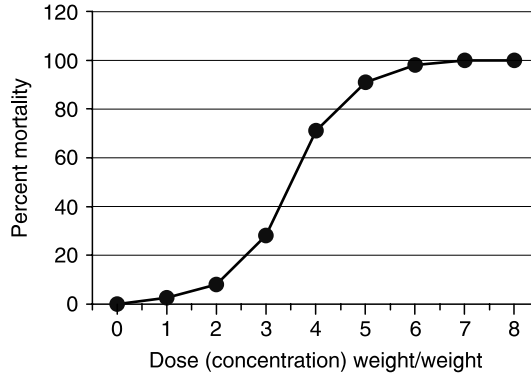

Figure 1.4 Plot of cumulative mortality vs. environmental concentration or dose. The data are plotted as cumulative number of deaths by each dose based on the data presented in Table 1.1.

- IC_{50} – IC stands for *inhibitory concentration*; IC_{50} causes a 50% decrease of the normal response of a test organism, estimated by graphical or computational means. IC_{50} is often used to measure effects such as the growth rate of seedlings, algae, and other organisms.

The toxicity of a compound is usually cited as the midpoint value, reported as a mass, per unit mass (mg/kg) or volume (mg/l). This practice is misleading and can lead to a misunderstanding or the true hazard of a compound to a particular xenobiotic. Conversely, compounds may have different LC_{50}, but the slopes may be the same. Similar slopes may imply a similar mode of action.

Toxicity is not generated by the unit mass of xenobiotic, but by the molecule. Therefore, molar concentration or dosage provides a more accurate assessment of the toxicity of a particular compound. Another weakness of LC_{50}, EC_{50}, and IC_{50} is that they reflect the environmental concentration of the toxicant over the specified time of the test. Compounds that move into tissues slowly may have a lower toxicity in a 96-hour test simply because the concentration in the target tissue has not reached toxic levels within the specified testing time.[4]

Other terminology is used to describe concentrations that have a minimal or nonexistent effect. Those that are commonly used include:

- NOEC – *No observed effects concentration* determined by hypothesis testing.
- NOEL – *No observed effects level* determined by statistical hypothesis testing methods. This parameter is reported as a dose.
- NOAEC – *No observed adverse effects concentration* determined by statistical hypothesis testing methods. The effect is usually chosen for its impact on the species tested.
- NOAE – *No observed adverse effects* level, determined by statistical hypothesis-testing methods.
- LOEC – *Lowest observed effects concentration*, determined by statistical hypothesis-testing methods.

- LOEL– *Lowest observed effects level*, determined by statistical hypothesis testing methods.
- MTC – *Minimum threshold concentration*, determined by statistical hypothesis-testing methods.
- MATC – *Maximum allowable toxicant concentration*, determined by graphical or statistical methods.

These concentrations and doses usually refer to the concentration or dose that does not produce a statistically significant effect. The ability to determine accurately a threshold level or no-effect level is dependent upon a number of criteria, including:[4]

- Sample size and replication
- Number of endpoints observed
- Number of dosages or concentration
- Ability to measure the endpoints
- Intrinsic variability of the endpoints within the experimental population
- Statistical methodology

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1.7 REVIEW QUESTIONS

1. What is the principal objective of the study of environmental toxicology?
2. What are the most marked developments in our environment in the recent decades?
3. What does NEPA stand for?
4. What is meant by toxicity?
5. Define point sources and nonpoint sources.
6. What is the dose–response relationship? What is its importance in our study?

7. Briefly define the following:
   a) LD_{50}
   b) LC_{50}
   c) EC_{50}
   d) NOAEC
   e) NOEL

Chapter 2

Environmental Change and Health

2.1 OUR CHANGING ENVIRONMENT

2.1.1 Introduction

The environment, which sustains the life of all living organisms, can also be a significant cause of ill health. As discussed in the previous chapter, increasing industrialization, expanding technology and economics, coupled in recent decades with growing world population, have radically changed, and are still changing, our environment. Some of the marked changes include global climate changes, increased air and water pollution, acid rain, mounting quantities of solid waste, destruction of the ozone layer by chlorofluorocarbons (CFCs), and the presence of a growing number of endocrine disrupters in the environment. These changes have profound impacts on the health and well-being of living organisms.

Literature dealing with some of these issues abounds. For example, *Time* magazine, in a rare departure from its tradition of naming "Man of Year", designated "Endangered Earth" as "Planet of the Year" for 1988. The January 2, 1989 issue of the magazine was dedicated to this particular theme. In the front section, which contained several articles on the issue, are these words: "What On EARTH Are We Doing?"[1] In this chapter, several issues of concern are discussed.

2.1.2 Global Climate Changes

Global climate changes, particularly global warming, have attracted much attention in recent years. According to studies by the National Oceanic and Atmospheric Administration (NOAA), over the period 1978 to 2002 the global tropospheric temperature increased 0.22 to 0.26°C per 10 years. The increase was consistent with the global warming trend derived from observations by surface meteorological stations.[2]

According to a recent report by the *New York Times*, researchers have found that the icecap atop Mount Kilimanjaro in Tanzania is retreating at such a pace that it will disappear in less than 15 years. The vanishing of the seemingly perpetual snows of Kilimanjaro echoed similar trends on ice-capped peaks in various parts of the world, including Canada and Peru, and is considered one of the clearest signs that recent global warming appears to have exceeded typical climate shifts. Measurements taken on Kilimanjaro show that its glaciers are not only retreating but also rapidly thinning, with one spot

having lost approximately 1 m of thickness since early 2002. Some scientists indicate that the mountain has lost 82% of the icecap it had in 1912, when it was first carefully surveyed.

Climate changes have also been shown to affect ocean temperature, salinity and flow patterns. Warmer temperatures weaken the ice, making it vulnerable to current changes and other forces. Some scientists consider that this has already influenced the stability of ice shelves in the Antarctic. Indeed, two chunks of ice the size of a small country broke off from the Antarctic Peninsula's Larsen Ice Shelf in 1995 and 2002.[3]

Scientists in the U.S. and Canada have observed a similar phenomenon occurring in the Arctic. They report that the largest ice shelf in the Arctic, a solid feature for 3000 years, has broken up. The report shows that the Ward Hunt Ice Shelf, on the north coast of Ellesmere Island in Canada (the northernmost land mass of North America), broke into two main parts, themselves cut through with fissures. Only 100 years ago, the whole northern coast of Ellesmere Island was edged by a continuous ice shelf. According to the report, about 90% of the shelf is now gone. Records indicate an increase of 0.4°C every 10 years since 1967. The average July temperature has been 1.3°C since that year.[4]

Environmental researchers believe that the burning of fossil fuels is slowly causing the climate to change. Exhaust from burning these fuels increases the level of carbon dioxide (CO_2) and nitrogen oxides (NO_x) and particulate matter in the atmosphere. This, in turn, causes the earth to retain heat, warming the globe. The CO_2 level in the atmosphere is already dangerously high. According to a recent report by the Intergovernmental Panel on Climate Change, an atmospheric CO_2 level of 540 to 970 ppm and a global temperature rise of 1.4 to 5.8°C could occur by 2100. Some scientists are concerned about an even more worrisome effect on future generations. With the long residence time of CO_2 in the atmosphere and warmer oceans, what are the prospects for the 22nd century? Many scientists consider that, because of their wealth and advanced technology, the U.S. and other industrial nations may be able to cope with the effects of global warming in their own countries in this century, but are unlikely to escape serious impacts in the following century.[4]

Knowledge about the contribution of CO_2 and other greenhouse gases to global warming has led a number of countries to reduce their emissions. This trend is particularly marked in several European countries, such as Germany, France, Italy, and the U.K. By contrast, some Asian countries, including China, India, and South Korea, have markedly increased their energy-related carbon emissions over the past two decades (Figure 2.1).[5] The U.S. General Accounting Office, which released the report, also predicts that China's emissions, now equivalent to half the U.S. output, will reach more than 80% of U.S. output by 2025.[5]

An often-debated question is the impact of increased CO_2 levels on vegetation. Some laboratory studies indicate that the rise of CO_2 levels in the atmosphere will stimulate plants to grow more abundantly, but others suggest that is not necessarily the whole story. New research in California has found

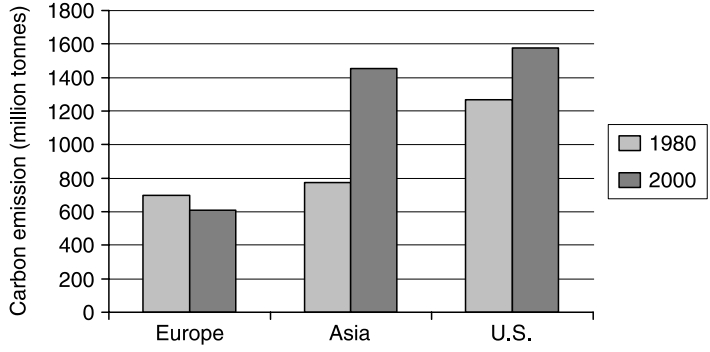

Figure 2.1 Comparison of greenhouse gas emission in various countries.
Note: "Europe" includes France, Germany, Italy, and U.K.; "Asia" includes China, India, Japan, and South Korea.

that when other elements linked to global climate change are added to the environment of plants, CO_2 actually may impair growth. Other researchers state that the effects of CO_2 can be either good or bad, depending on certain other elements of the environment.

Another concern about the impact of global warming is the possible resultant rise in diseases. For instance, serious diseases broke out in several countries during the 1990s after extraordinary heat followed by various extreme weather conditions, such as heavy monsoons and floods. Significant numbers of deaths occurred worldwide, resulting from diseases such as cholera, pulmonary hantavirus, plague, and dengue fever. Some scientists caution that perhaps even more immediate threat of the warming trend is the rapid spread of disease-bearing insects and pests.[6]

2.1.3 Air Pollution

2.1.3.1 Introduction

Air pollution can be defined as the presence of substances in air at such concentrations, duration, and frequencies that adverse effects on the health of living organisms and the environment may be caused. For several decades, concerns over air-pollution problems have increased steadily since the end of World War II, particularly in the more-developed countries. The extent to which air pollution influences public health is shown by many air pollution-related episodes. One of those episodes is the widely known 4000 "excess deaths" that occurred in London in 1952. Similar but less serious air-pollution-related injuries have also occurred in other major cities in the world, including Osaka, Los Angeles, and New York, although the air pollutants involved were often different from one another.

A wide range of pollutants are present in indoor and outdoor air. They include sulfur oxides (SO_x), NO_x, carbon monoxide (CO), ozone (O_3) and

other photochemical oxidants, different types of particulates, lead and other heavy metals, and various kinds of volatile organic compounds (VOCs). The major source of air pollution is the combustion of fossil fuels for electricity and transportation, various industrial processes, heating, and cooking. According to the North American Commission for Environmental Cooperation (CEC), one-quarter of the industrial pollution released into the North American environment in 1998 came from U.S. electric power plants. This is closely followed by pollution from the primary metals sector, the chemical industry, and the hazardous waste management sectors.[7]

2.1.3.2 Air Pollution and Developing Economies

While problems associated with air pollution remain of global concern, encouraging results were shown for its control in the U.S. and other industrialized countries. For example, according to a recent EPA report, a large improvement in air pollution has occurred in the U.S. since 1970. Emissions of six principal air pollutants (i.e., SO_x, NO_x, CO, O_3, particulate matter, and lead) have declined 48% since 1970. Sulfur dioxide (SO_2) emissions from power plants are 9% lower than in 2000, and 41% lower than in 1980, while NO_x emissions declined 13% from 2000, and 33% from the 1990 level. The levels of ground-level O_3, however, have decreased the least. The ten-year trend has been relatively unchanged.[7]

By contrast, many of the rapidly growing cities in the world are experiencing an increasing number of environmental problems, especially those related to air pollution. Serious concerns have been raised about the health hazards of air pollution in a number of less-developed countries. With unprecedented growth shown in urban centers, megacities with populations of 10 million or more have emerged in many less-industrialized countries, including China and India. In India alone there are four such cities, with three others expected to join the ranks in the next 20 years.[8] In India, a majority of the 300 million urban dwellers, who constitute 30% of India's population, are experiencing deteriorating air quality. Major cities in India are reportedly among the most polluted in the world, with concentrations of several air pollutants well above the levels recommended by the World Health Organization (WHO). Some scientists in the country caution that the residents of India's megacities face significant risks to their health from exposure to air pollutants.[8]

As is widely known, China has achieved rapid economic growth during the past several decades. The growth is coupled with industrialization, accelerated urbanization, and greatly increased energy consumption.[9] The accelerated urbanization is evidenced by marked increases in the proportion of urban population to the total population in China, from 18% in 1978 to 31% in 1999, a growth rate three times the world average during this period. The explosive economic growth also made China the world's second-largest energy consumer, after the U.S. Energy consumption, especially coal consumption, is the main source of anthropogenic air-pollution emissions in Chinese cities.

Between 1978 and 1999, China's energy consumption more than doubled. Coal, the primary energy source in China, accounted for about 74% of the total energy consumption during this period. It is considered that the use of coal is the origin of many air-pollution problems, such as SO_2 pollution, particulate matter, and acid rain.[9]

Furthermore, consumption of crude oil has also increased, with the average rate of increase of 6% per year in the past decades. Part of this increase is the result of the growing use of motor vehicles, which has raised the ambient pollution by NO_x, CO, and related pollutants in large cities. Indeed, China's growing energy consumption, reliance on coal, and rapidly increasing use of vehicles place a heavy burden on urban atmospheres in the country, and urban air pollution has been rapidly emerging as a major environmental issue. Many Chinese cities have suffered from increasingly serious air pollution since the 1980s. During the 1990s, some megacities, such as Beijing, Shanghai, Shenyang, and Guangzhou, were always listed among the top 10 most-polluted cities in the world.[9]

Some researchers express serious concerns about the public health effects of urban air pollution in China.[9] The concerns were strongly supported by the studies of Xu et al.,[10] whose study led them to conclude that the existing air-pollution levels in Beijing are associated with adverse health outcomes. The scientists studied the data on the average number of daily hospital outpatient visits at a community-based hospital in Beijing, and compared the data with the levels of SO_2 and total suspended particles (TSPs) in the atmosphere. They found that increases in the levels of the two types of pollutants were significantly correlated with increases in hospital visits relating to internal medicine, in both winter and summer.[10]

Similar observations have been made in Seoul, South Korea, where a number of scientists have investigated the impact of air pollution on human health. For example, Ha et al.[11] studied the effect of air pollution on mortality among postneonates, people aged 2 to 64 years, and those over 65 years of age. The study included daily counts of total deaths and deaths due to respiratory problems, along with analyses of daily levels of atmospheric particulate matter less than 10 μm in diameter (PM_{10}). The results showed, as expected, that infants were most susceptible to PM_{10} in terms of mortality, particularly mortality related to the respiratory system.[11]

2.1.4 Water Pollution

Historically, the concern about water pollution was a concern about its health effects. While in many countries this remains true, in the U.S. and other developed countries, the results of improved treatment and distribution methods have, to a large degree, shifted the emphasis. Many citizens in these countries generally regard water pollution not so much as a health issue, but rather an issue of conservation, aesthetics, and the preservation of natural beauty and resources. Nevertheless, many of the world's lakes, rivers, and streams have suffered, and are still suffering, from the effects of water

pollution. Moreover, the problems associated with water pollution are worsening in many countries, particularly in some of the less-developed ones.

The main sources of water pollution include both inorganic and organic wastes, heat from industries, petroleum compounds, municipal wastes, agricultural wastes, pesticides, and acid mine drainage. Many industrial processes have the potential to discharge various types of wastes that could cause significant water pollution problems.

Human diseases and casualty arising from water pollution attracted worldwide attention after "Minamata disease" and "itai-itai-byo" ("ouch-ouch disease"), which occurred in Japan during the 1940s and 1950s. Minamata disease was caused by eating fish and shellfish laden with highly toxic methylmercury, while itai-itai-byo was mainly attributed to ingestion of rice contaminated with high levels of cadmium. (More-detailed information on heavy metals is presented in Chapter 12.)

In addition to heavy metals, a variety of inorganic and organic compounds can also contaminate streams, lakes, and rivers, threatening their water quality. The recent observation that stream water, and also garden fertilizers, may be contaminated with perchlorate is an example. Industrial and military operations and fireworks manufacturers use perchlorate as an oxidizing agent, and they appear to be the primary sources of contamination.[12] Perchlorate is potentially harmful to thyroid function, and could be widespread in some American agricultural areas – earlier studies by the EPA research laboratory indicated that common garden fertilizers contained perchlorate concentrations up to 0.84% by weight. However, a subsequent study released in June 2001 by the same agency showed that the majority of fertilizers used in the U.S. are not contaminated with perchlorate salts.[12]

Water pollution can not only influence human health directly, but also threaten aquatic life, particularly fish. For instance, in the early 1960s, millions of fish in the lower Mississippi River died from the effects of chlorinated organic pesticides, particularly endrin. In the early 1970s, contamination of fish by DDT and polychlorinated biphenyls (PCBs) caused an abrupt halt to commercial salmon fishing in the upper Great Lakes.

Although much progress has been made since, and the public is encouraged by the reports on the decreased levels of chlorinated hydrocarbons and other toxicants in fish crops, problems of water pollution in Great Lakes appear to persist, as seen in Case Study 2.1. Case Study 2.2, however, shows that pollution problems can be reversed given the right conditions.

Case Study 2.1

The Detroit News recently published an eye-opening report, under the title "Disappearing shrimp pose threat to Great Lakes whitefish." According to the report, one of the principal food sources for whitefish is disappearing rapidly from the Great Lakes, a change that threatens to shake up the food chain and impede the state of Michigan's large commercial fishing industry. The report shows that diporeia (*Diporeia* spp.), shrimp-like creatures about 12 mm in

length (sometimes referred to as fresh-water shrimp) that live on the bottom of the Great Lakes, have been wiped out in portions of Lake Erie, Lake Michigan, Saginaw Bay, and Lake Ontario. About 44,000 km^2 of the Great Lakes no longer have diporeia. Research biologists indicated that they have never seen such a phenomenon before. In the 1980s, the scientists found densities of diporeia between 3860 and 7720 per km^2 of sediment in parts of the Great Lakes. The researchers state that no diporeia are now found in many of the same spots. Diporeia are a main food source for many fish in the Great Lakes. Whitefish have become one of the first casualties of the loss of diporeia. Until recently, whitefish could be found that were about 0.6 m long and 2.3 kg. Now whitefish range from 0.51 to 0.56 m. The decline of the diporeia population remains somewhat of a mystery to fish researchers. They have examined whether the decline is a result of contaminants, but, so far, there is no conclusive answer.

Case Study 2.2

Around the middle of the 1960s, New York City's Hudson River was found to be "dying" as a result of severe water pollution. The sources of the pollution were found to be raw sewage being dumped into the river by the city; discharge of large quantities of paint from a factory; oil dumping from Penn Central Railroad; and discharge of water at elevated temperatures from a nuclear power plant. There is, however, reason to be encouraged. In 1966, several fishermen formed the Hudson River Fishermen's Association. Mainly because of their efforts and those of others who joined subsequently, much improvement has been made. Beginning in 1968, a number of polluters were forced to spend millions of dollars remediating the Hudson. The by-product of these actions was one of the greatest environmental success stories of the 20th century. Today, the Hudson produces more fish per hectare than most other major estuaries of the North Atlantic. Fish and fishermen, boaters, and swimmers have reportedly returned to the river.[13]

2.1.5 Soil Pollution

Another major concern is the possible deleterious effect of the release of an increasing number of toxic synthetic chemicals into the environment. This leads to soil pollution, in addition to air and water pollution, and food contamination. Moreover, the release of these chemicals is not limited to areas adjacent to point sources, such as industrial facilities. Rather, the chemicals can be transferred to distant areas and regions where they may elicit adverse effects on living organisms.

In the U.S., an assessment of the extent and severity of contamination is further complicated by the nearly exponential growth of the synthetic organic chemicals industry since the early 1940s. About 70,000 chemicals are estimated to be in common industrial and commercial use in the U.S. and this number continues to grow by about 1000 new compounds every year. Only a limited number of ecological assessments on the bulk of the chemicals on the market or those introduced each year have been undertaken. The human health effects of

many of these chemicals, particularly over long periods of time at low exposure levels, is largely unknown.

One of the most widely known episodes related to disposal of hazardous wastes is that of Love Canal, an abandoned canal bed near Niagara Falls in the state of New York (see Case Study 2.3).

Case Study 2.3

In the1940s and 1950s, Hooker Chemical & Plastics Corporation dumped over 23,000 t of chemical wastes into the Love Canal landfill.[14] After the canal was filled and covered with earth, the land was transferred to the city of Niagara Falls. Homes and a school were then built on the edge of the old canal and the area of covered chemicals became a playground. In 1968, Occidental Chemical (OxyChem) purchased Hooker Chemical Company. In 1977, black oily fluids oozed from the ground in the vicinity of the canal. The fluids were subsequently identified as a mixture of potent chlorinated hydrocarbons. Children attending the school showed unusual health problems, such as skin rashes, chemical burns, and severe physiological and nervous disorders. Furthermore, unusually high numbers of miscarriages and birth defects were noted. A lawsuit amounting to nearly $3 billion in health claims was then filed against the city of Niagara Falls. Eventually, the state purchased and demolished about 100 homes in the area and state officials evacuated 500 houses in 1978. Federal and state crews cleaned up the landfill and surrounding contaminated areas. Litigation followed between New York State and OxyChem. In 1994, OxyChem and the state finally agreed to settle their conflicting claims stemming from the incidence. (Remediation of the land eventually took place, followed by resettlement of the area. By 1994, nearly 70% of the 280 available houses had been sold. A survey showed that about 30% of the purchasers had been residents in the area before the evacuation.)[14]

2.2 THE CHANGING DISEASE PATTERN

Associated with the changes in the environment are the changing pattern and distribution of diseases or health effects. For instance, at the turn of the century, pneumonia and tuberculosis were the two leading causes of death in most countries, including the U.S. Because of improved sanitation and public health measures, coupled with advancement in medicines and technology, tuberculosis and other contagious diseases have largely been eradicated. In place of these relatively straightforward illnesses, however, are diseases that are more complex and have multiple causes, including chronic heart diseases, chronic respiratory diseases, and malignant neoplasms or cancers. It is widely known that, since about 1950, cancer and diseases of the heart have become the two leading causes of deaths in the U.S. Importantly, these diseases, as well as chronic lower respiratory diseases and chronic liver disease and cirrhosis, are considered environmentally related (Table 2.1).[15]

Table 2.1 Changing Causes of Death in the U.S. between 1950 and 2000

	Year					
	1950		1980		2000	
Rank	Cause of death	%[a]	Cause of death	%[a]	Cause of death	%[a]
1	Disease of heart	40.5	Disease of heart[b]	39.6	Disease of heart[b]	29.6
2	Malignant neoplasm	13.4	Malignant neoplasm[b]	20.0	Malignant neoplasm[b]	22.9
3	Cerebrovascular diseases	12.5	Cerebrovascular diseases	9.2	Cerebrovascular diseases	7.0
4	Unintentional injuries	5.4	Unintentional injuries	4.4	Chronic lower respiratory diseases[b]	5.1
5	Influenza and pneumonia (chronic nephritis)	3.3	Influenza and pneumonia	3.0	Unintentional injuries	4.0
6	Diabetes mellitus	1.6	Chronic lower respiratory diseases[b]	2.7	Diabetes mellitus	2.8
7	Suicide	0.9	Diabetes mellitus	1.7	Influenza and pneumonia	2.7
8	Chronic liver disease[b]	0.8	Chronic liver disease[b]	1.4	Suicide	1.2
9	Chronic lower respiratory diseases[b]	0.5	Suicide	1.1	Chronic liver disease[b]	1.1
10	Homicide	0.3	Homicide	1.0	Homicide	0.6

[a] Percent of total deaths from all causes.
[b] Diseases that are considered environmentally related.
Source: USDHHS, *Health, United States, 1996–97 and Injury Chartbook*, 1997; USDHHS: *Health, United States*, 2003.

The above-mentioned changes in disease patterns have also been observed in many other countries, including the less-developed world. For example, in Brazil in 1940, infectious diseases caused 39 to 60% of all deaths, depending on the region of the country, but by 1980 these diseases accounted for only 3 to 16% of deaths. However, cardiovascular diseases accounted for only 9 to 13% of mortality in 1940 but rose to 20 to 38% in 1980.[16]

What are the reasons for these changes? Scientists consider that environmental pollution may play a role in such a shift. Environmental pollution affects all living organisms, including humans. Many human diseases are traceable to substances in the air, water, and the foods we consume. Some of the industrial agents released into the general environment are also known to be, or suspected of being, carcinogenic (cancer-causing).

2.3 EXAMPLES OF ENVIRONMENTAL DISEASES

2.3.1 Introduction

Many diseases have long been recognized as being related to occupation. The British doctor Percivall Pott is widely recognized as being the scientist who, in 1775, first pointed out the direct connection between an occupational exposure and the risk of a specific cancer, i.e., chimney sweeps and cancer of the scrotum.[17] Miners, stone cutters, and lens grinders often developed respiratory disease from inhaling large quantities of dust. Many hatters suffered brain damage as a result of absorbing highly toxic vapors from mercurials (chemical compounds containing mercury) used in making felt. Asphalt, coal tar and pitch workers, textile dyers, and shoe and leather workers are all suspected of having an increased risk of developing bladder cancer because of their association with coal products and aromatic amines.

However, in the past several decades, environmental diseases have spread beyond those employed in a few specialized occupations.[18] Among the most serious are cancer, respiratory diseases, birth defects, heavy-metal poisoning, and injury to the reproductive system. These are briefly discussed in this chapter, and are covered in more detail in subsequent chapters.

2.3.2 Cancer

Many researchers recognize that a close association exists between industrial activities and cancer incidences and cancer death rates. The U.S. has one of the world's highest incidences of cancer associated with environmental pollution. Since about 1950, cancer has been second only to heart disease as the cause of death in the U.S. Moreover, until recently the rate of cancer deaths had been increasing steadily (Table 2.1 and Table 2.2).[19] The actual number of deaths from cancer is still rising, for example 416,509 Americans died of cancer in 1980, but by 1990 the figure had increased to 505,322, and in 1999 it was

Table 2.2 Cancer Death Rates between 1950 and 2000 in U.S. Age-Adjusted Death Rates per 100,000 Population

Year	1950	1960	1970	1980	1990	2000
Deaths from all causes	1446	1339.2	1222.6	1039.1	938.7	869.0
Total cancer deaths	193.9	193.9	198.6	207.9	216.0	199.6
%	13.41	14.48	16.24	20.00	23.01	22.97
Percent increase/decrease over previous decade	–	7.98	12.15	23.15	15.05	−0.21
Deaths from respiratory-system cancer	15.0	24.1	37.1	49.9	59.3	56.1
%	1.04	1.80	3.03	4.80	6.31	6.45
Percent increase/decrease over previous decade	–	73.07	68.33	58.41	31.45	2.21

Source: Data from USDHHS, *Health, United States, 2003.*

549,838.[19] According to American Cancer Society, the estimated toll for 2003 was 556,500[20] – more than 1500 deaths per day.

The northeast region of the U.S. is known as a highly industrialized and polluted area. This region also is known to have a particularly high incidence of cancer. Studies carried out by the National Cancer Institute indicated that areas close to the locations of iron and lead smelters have high rates of lung cancer. Studies show that nearly 30% of the total mortality in several industrialized countries is due to cancer.[21] Cancer incidence and mortality in most of these countries have been consistently increasing in recent decades. In particular, this trend is independent of the aging of the population.

In humans, the main sites where cancers develop include the brain and nervous system, breast, colon and rectum, blood (leukemia), liver, lung and bronchus, lymphatic system (Non-Hodgkin's lymphoma), ovary, pancreas, and prostate.[19] Environmental factors (such as lifestyle, personal habits, diet, chemicals and radiation, and infectious diseases) account for about three quarters of all cancers. According to the American Cancer Society,[20] smoking, obesity, and physical inactivity have a greater effect on individual cancer risk than do exposure to trace amounts of pollutants in air, food, or drinking water. However, the degree of risk from pollutants depends on the concentration, intensity, and duration of exposure. Substantial evidence exists showing significant increases in cancer risk in settings where workers have been exposed to high levels of certain chemicals, such as heavy metals and organic compounds, as well as from radiation.

As mentioned above, in the past 100 years, and particularly since World War II, as a result of accelerating industrial development, a large number and quantity of chemicals have been released into the environment. The release has led to increased pollution of the air, water, and soil, potentially contaminating food sources. Areas with industrial plants that manufacture soaps, rubber, chemicals, and printing inks have high rates of bladder and liver cancer. A New York Department of Health study has found that, in Nassau County, women living within 1 km of a chemical, petroleum, rubber, or plastics facility were 60% more likely to develop postmenopausal breast cancer than were those who lived in other parts of the country.[21]

An alarming trend associated with cancer is the high incidence rate among children in the U.S. An estimated 9000 new cases, and 1500 deaths, were expected to occur among children aged 0 to 14 in 2003. About 30% of the deaths are likely to have been from leukemia. Despite the rarity of childhood cancer in the U.S., it is the chief cause of death by disease in children between ages 1 and 14.[20] According to the National Cancer Institute, the rate of increase has amounted to nearly 1% a year. Some experts in the field estimate that a newborn child today faces a risk of about 1 in 600 of contracting cancer by the age of 10. Although the reason for the high incidence rate of childhood cancer in the U.S. remains unclear, some scientists suspect that exposure to environmental pollutants by the pregnant mother or the children may be an important factor. An encouraging piece of information was recently given by

the American Cancer Society, indicating that the mortality rates of childhood cancer have declined by about 47% since 1975.[20]

The association of pesticides and related chemicals with various illnesses and death has attracted a wide attention and much study. Of particular concern are chlorinated hydrocarbon-based pesticides and dioxin. For instance, accidents during the manufacture of the herbicide 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and polychlorinated phenol derivatives have caused acute dioxin poisoning of plant workers and populations in several countries.

As is widely known, 2,4,5-T and related dioxin-contaminated defoliants were used extensively in Vietnam from 1961 to 1969. Among the major toxic effects attributed to dioxins is liver cancer. Between 1956 and 1961 (the year in which spraying of the herbicides began), 159 cases of primary hepatic cancers were recorded among 5492 cancers in the Hanoi area, while between 1962 and 1968, 791 primary hepatic cancers were observed in a total of 7911 cancers. This change represented a more than three-fold increase in the proportion of primary cancer of the liver.[22]

2.3.3 Birth Defects

It is estimated that approximately 3% of all live births in the U.S. have significant birth defects.[23] This represents about 100,000 congenital anomalies in a total of 3 million live births annually. Congenital malformations are the leading cause of infant mortality in the U.S. Furthermore, studies show that the presence of any malformation diagnosed during the first year after birth increased mortality 18-fold for white infants. Clearly, enormous financial costs and emotional suffering are associated with these malformations.

The etiologic nature of the majority of congenital malformations in infants is largely unknown. It has been estimated that about 5 to 10% of all birth defects are due to an *in utero* exposure to a known teratogenic agent or maternal factor. Intrauterine growth retardation can be caused by a number of agents, including hypoxia (a deficiency of oxygen reaching the tissues of the body), drugs, x-ray irradiation, maternal endocrine and nutritional factors, and environmental chemicals. Many chemical species are known to be teratogenic, i.e., capable of causing birth defects. These chemicals include various organic solvents, pesticides, dioxins, several heavy metals (such as lead, cadmium, and mercury), and others. Many human epidemiological data support the claim that environmental chemicals are an important factor responsible for inducing teratogenicity.

2.3.4 Reproductive Damage

An increasing number of studies have shown that a variety of toxicants can induce detrimental effects on reproductive systems in animals and humans. For instance, reproductive damage in seagulls and other wildlife presented some of the first clues about the adverse effects of DDT.[24] Organochlorines have also

been implicated in impaired reproductive success in fish populations of the Baltic Sea[25] and the North Sea.[26] These compounds also have detrimental effects on the health and reproduction of seals.[27,28,29]

More recently, reproductive anomalies in wildlife have sparked concern about the ability of a number of chemicals to cause ill effects by disrupting the body's normal hormonal system. An increasing number of chemicals are now known to have such action. Examples include organochlorines, such as PCBs, dioxins, as well as DDT; pesticides such as carbamates (e.g., aldicarb, carbofuran), triazines (e.g., atrazine and simazine), and pyrethroids, (see Chapters 3 and 12); heavy metals such as cadmium, lead, and mercury; and organobrominate compounds.

The reproductive toxicity of the pesticide 2,2-dibromo-3-chloropropane (DBCP) became clear in the late 1970s and early 1980s when male farm workers in the banana-growing region of Costa Rica were found to be sterile. By the mid-1990s, nearly 1500 male workers had been diagnosed with sterility from exposure to DBCP.[30]

There has been a steady rise in the number of premature births in the U.S. According to U.S. government statistics, 11.8% of all babies (about 440,000 infants), were born prematurely in 1999 – that is, before the end of the 37th week of gestation (the normal length of gestation is 40 weeks). According to data from the National Center for Health Statistics, in 1981 9.4% of live births were premature. Although strong evidence is still lacking, some researchers presented data at a meeting in October 2001, sponsored by the Institute of Medicine, suggesting that industrial chemicals, pesticides, and air pollutants could have contributed to the 23% rise in premature births in the U.S. since the early 1980s. One of the strongest associations was found in a study that measured the levels of DDE (a metabolite of DDT) in stored sera of mothers who gave birth between 1929 and 1966, when DDT was heavily used in the U.S. In a sample group of 2380 babies born to these women, 361 were preterm and 221 were small for gestational age. The greater the level of DDE in the mother's blood, the higher was the risk for the infant.[31]

Shortened gestation times were also reported to be associated with benzene exposure. A Chinese scientist studied 542 births to women working at a petrochemical plant in Beijing, and found that benzene shortened the pregnancies of those women who had a genetic profile that prevented them from detoxifying benzene easily.[32] The health effects of benzene are discussed in more detail in Chapter 11.

2.3.5 Respiratory Diseases

Many epidemiological and animal studies have shown that airborne pollutants are commonly found in the urban environment in concentrations high enough to adversely affect the lungs.[33] During the past five decades, chronic bronchitis, emphysema, and lung cancer have become major public-health problems in the U.S. and other major industrialized countries. In the U.S., although heart diseases have been known as the number one killer for several decades, death

rates for the diseases increased between 1950 and 1960, but have since declined steadily. For example, expressed as percentage of total death rate, the death rates of heart diseases were 41.7%, 39.6%, and 29.7% for 1960, 1980, and 2000, respectively.

By contrast, the cancer death rates in the U.S. continued to increase steadily until the middle of the 1990s, when the increase began to slow down.[19] In particular, the death rates for respiratory-system cancer increased dramatically over the past five decades. Using the 1950 rates as a basis for comparison, the respiratory-system cancer death rates increased by 191% and 506% for 1970 and 1990, respectively (Table 2.2). By contrast, the increases in cancer deaths from all causes were 21% and 71% for 1970 and 1990, respectively. The marked differences in both categories of cancer death rates are more clearly shown in Figure 2.2. While the reasons for the differences are not entirely known, it is possible that exposure to increasing levels of air pollution may play an important role.

In Japan, the level of air pollution has markedly decreased since the early 1970s, but the number of patients with respiratory disease due to air pollution has increased. Between the late 1950s and 1960s, a large number of patients in Japan suffered from chronic obstructive lung diseases such as chronic bronchitis, bronchial asthma, and emphysema. Studies showed that, during this period, there were many chronic-bronchitis patients in Yokohama and Kawasaki, two highly industrialized cities near Tokyo that were heavily polluted with SO_2 and soot. Researchers in Japan concluded that the SO_2 pollution caused acute respiratory diseases and aggravated the condition of patients already suffering from respiratory disease. One of these respiratory conditions was even referred to as "Yokohama and Kawasaki Asthma."[34]

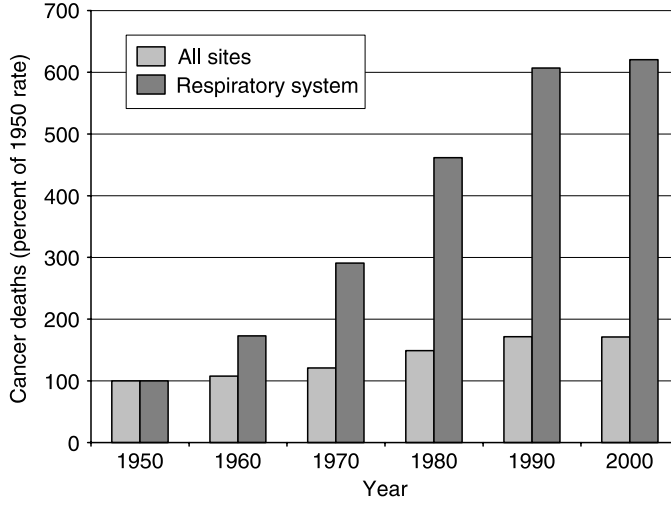

Figure 2.2 Comparison of death rates for cancer of all sites vs. cancer of respiratory system between 1950 and 2000.

2.3.6 Heavy-Metal Induced Diseases

Following the Industrial Revolution, the production of heavy metals, such as copper (Cu), lead (Pb), and zinc (Zn), has increased dramatically. Between 1850 and 1990, the production of these three metals rose nearly tenfold, with concomitant increases in the emission of various metals including cadmium (Cd), mercury (Hg), and nickel (Ni).[35] Another toxic element is arsenic (As). Because of industrial pollution, some of these metals and nonmetallic elements accumulate within limited geographic areas to excessive levels, which have produced major outbreaks of chronic illness in humans. Some notable examples of heavy-metal induced diseases and poisoning incidents follow.

Although chronic Pb poisoning has plagued humans since at least the time of the ancient empires, the importance of Pb as an environmental pollutant has received widespread attention only in recent decades. In ancient Rome, Pb in pipes and in drinking and cooking vessels was a major source of excessive intake. Even today, Pb contamination in water supplies occurs in some communities. Lead pipes in older plumbing and soldered pipe joints can contaminate drinking water, especially "soft" water. However, the Pb in smoke from burning trash and coal and, until recently, automobile exhausts, is probably even more hazardous as it is inhaled, or ingested as a contaminant of foods (after settling on vegetation).

Lead-based paint in older homes is even more dangerous because small children often ingest paint from woodwork, plaster, floors, and furniture. It is not surprising, therefore, that as many as 25% to 30% of American children living in urban areas may be suffering from "subclinical" Pb poisoning.[36] The most prominent adverse effects of Pb involve the nervous system, the hematopoietic system (the organic system of the body consisting of the blood and the structures that function in its production), and the kidneys.

As mentioned previously, one of the most serious outbreaks of anthropogenic poisoning of the industrial age is the epidemic of Hg poisoning, now known as "Minamata disease." This illness occurred in Minamata Bay in Kyushu, Japan, in 1953, and the highest incidence was found to be among fishermen and their families.[37] Later, when it was observed that household cats and sea birds were being affected, attention turned to fish and shellfish as etiologic factors. This in turn led to the study of the water of Minamata Bay and to the identification of Hg in a factory effluent as the cause of the disease. The study concluded that fish that had been consumed by sufferers contained high levels of toxic methylmercury (MeHg). When ingested, MeHg can induce permanent damage to the brain and kidneys, loss of vision, and disturbed cerebral function. Ultimately, coma and death follow in severe cases.

The discovery of gold (Au) in Serra Pelada in the Amazon in 1979 touched off a great flow of migrants into that area in the 1980s. There are potentially serious health effects from exposure to high levels of metallic Hg during mining of Au. Hg is used to bind the Au, and the resultant amalgam is heated at high temperatures with a blowtorch to separate Au from the Hg. This vaporized Hg gradually accumulates in the aquatic food chain. In contrast to the Hg

poisoning in Minamata, where a single industrial source polluted one local fishing area, in the Amazon region thousands of Hg sources pollute the waters. Brazilian mining agencies estimated that 300,000 miners had been distributed among 1800 gold fields in the Amazon in the early 1990s. By 1996, some 3000 t of Hg had been released into the environment, compared with 200 to 600 t dumped into Minamata Bay.

Another outbreak of chronic illness called "itai-itai-byo" or "ouch-ouch disease" occurred along the Jintsu River in northern Japan in the mid-1950s. Victims of this disorder suffered severe bone pains. Eventually, the victims' softened bones disintegrated under even slight pressure, leading to multiple fractures. Death also occurred, and this was attributed to kidney failure that developed during the course of the disease. Extensive research ultimately identified the culprit as Cd in rice grown near a Pb and Zn mining facility. Effluent from the mine used in irrigating the ricepaddy, combined with Cd-laden fumes, had polluted the cultivated rice. In addition to its effect on bones, Cd is also a nephrotoxin and can cause hypertension. A more detailed discussion of heavy metals is presented in Chapter 12.

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2.5 REVIEW QUESTIONS

1. Briefly explain the air pollution episode that occurred in London in 1952.
2. What is air pollution? What are its main sources?
3. What are the six principal air pollutants?
4. Briefly describe the relationship between developing economies and environmental problems.
5. What is "Minamata disease?"
6. What does "itai-itai-byo" or "ouch-ouch disease" refer to?
7. Explain the changes in water quality in New York City's Hudson River during the 1960s and the 1990s.
8. Briefly explain the Love Canal episode.
9. What is the most pronounced change in disease pattern in the U.S. between the turn of the century and 1950?
10. Name five of the leading causes of death in the U.S. that are considered environmentally related.
11. What is the recent trend in the incidence rate of children's cancer in the U.S.?
12. What does "teratogenic" mean? Name three chemicals that are teratogenic.
13. Briefly explain how environmental chemicals may be associated with the reproductive system.
14. Explain the differences between the total cancer death rates and the respiratory-system cancer death rates in the U.S. between 1950 and 1990.
15. In Question 14, what would you conclude by looking at the data presented?
16. What are the most prominent adverse effects of Pb poisoning?
17. What environmental problem exists in gold mining in the Amazon Basin?

Chapter 3

Occurrence of Toxicants

3.1 INTRODUCTION

A large number of pollutants are present in the environment, often in very large quantities. They arise from many sources and exposure to these pollutants may occur through a range of routes. For example, the ambient air in urban areas may contain sulfur dioxide (SO_2), carbon monoxide (CO), and nitrogen oxides (NO_x), as well as smoke and suspended particles containing metals and hydrocarbons produced mainly from coal or heavy-oil combustion by industries, power plants, and some households. Several pollutants are also found in the indoor environment. Some examples include CO arising from incomplete combustion of fossil fuels and tobacco smoke, lead (Pb) from paint used in old houses, and formaldehyde from insulation and wood preservatives and adhesives.

This chapter will focus on where and how certain pollutants may occur in the environment. This is followed by a brief review of major pollution episodes and disasters that have occurred in recent decades.

3.2 VISIBLE SMOKE OR SMOG

The presence of visible smoke or smog is a manifestation of air pollution. Smoke is composed of the gaseous products of burning carbonaceous materials made visible by the presence of small particles of carbon. The brownish to blackish materials emitted from the stack of an inadequately controlled coal-burning industrial plant, or from the chimney of a wood-burning home, are examples. Wood burning has become a common practice in many American homes, especially in winter. Burning wood in a well-insulated home, however, can lead to discomfort associated with indoor pollution. The problem associated with indoor air pollution is particularly serious in many villages in southern China, where indoor combustion of coal for cooking meals or drying vegetables is common.

Smog, on the other hand, is a natural fog made heavier and darker by smoke and chemical fumes. Smog is formed mainly as a result of photochemical reactions. In the presence of UV rays in sunlight, nitrogen dioxide (NO_2) is broken down into nitric oxide (NO) and atomic oxygen. Atomic oxygen can then react with molecular oxygen in the air to form ozone (O_3). A large number of chemical reactions may also occur among hydrocarbons or between hydrocarbons and NO, NO_2, O_3, or other chemical species in the

atmosphere, leading to the formation of numerous chemical species. Both NO and NO_2 are called *primary air pollutants*, as they are formed at the source of combustion or emission. Compounds produced from chemical reactions that occur after the primary pollutants are emitted into the atmosphere are called *secondary pollutants*. Examples of secondary pollutants include O_3, peroxyacyl nitrate (PAN), and some aldehydes and ketones (NO_2 can also be included as a secondary pollutant – see Chapter 8). Smog is composed of both primary and secondary air pollutants; it contains NO_2, O_3, and other photochemical oxidants and a large number of other chemical species.

Both smoke and smog cause reduction in visibility because light is scattered by the surfaces of airborne particles. They can both cause adverse effects on vegetation, animals, and humans.

Although Los Angeles is widely known for its smog, many large cities are suffering increasingly from similar problems. This is particularly true in some less-industrialized countries that have experienced unprecedented growth in recent decades. This growth has led to the emergence of a number of megacities, with populations of 10 million or more people. Globally, many rapidly growing cities are also known to be among the most polluted in the world. Residents in those cities are overwhelmed by environmental problems, especially those related to air pollution. Examples of such countries include China, India, Mexico, and Thailand. The megacities in these countries are experiencing concentrations of a number of air pollutants well above the levels recommended by the World Health Organization (WHO). For example, Mexico City, with an estimated population of more than 20 million, has been experiencing serious air pollution problems.

Shen-Chen, a rapidly growing city in southern China, is another example with air pollution problems, even though its population is only about one million. In the morning, visibility is often good: it is possible to see the green mountain to the southwest of the city, but in the afternoon smog often develops, resulting in poor visibility. Figure 3.1a and Figure 3.1b show contrasting views of the city.

3.3 OFFENSIVE ODORS

Malodors are often the first manifestation of air pollution. They are present in natural air, households, farms, sewage treatment plants, solid waste disposal sites, and in many industrial areas. Natural air may contain odors arising from a variety of sources. Decomposition of protein-containing organic matter derived from vegetation and animals can contribute to odors in the air.

Odors from cooking foods, such as fish, meat, and poultry, can contribute greatly to the odors sensed in a household. Fresh paints, fresh carpets, furniture polish, cleaning fluid, wood-burning fireplaces, and deodorants are some other examples. Cigarette smoking can also be an important cause of odors in public places, restaurants or households.

(a)

(b)

Figure 3.1 Smog development in Shen-Chen, China: (a) clear morning, and (b) afternoon smog.

Offensive odors may be detected in areas adjacent to industries, and vary according to the type of industries involved. Some examples of industrial sources of malodors include:

- pulp mills, which release hydrogen sulfide (H_2S), causing "rotten-egg" type odors
- oil refineries, due to H_2S and mercaptans
- some chemical plants, due mainly to use of aniline or organic solvents
- food processing plants
- iron and metal smelters, which emit acidic smells
- phosphate fertilizer manufacturing plants

3.4 AGRICULTURAL DAMAGE

Agricultural damage constitutes the major damage to vegetation caused by air pollution (discussed in more detail in Chapter 8). A widely known example is the destruction of forests by acid rain. Ample evidence exists attesting to this phenomenon in the U.S., Canada, and in some European countries. Acid rain causes changes in plant growth that are manifested by stunted growth, lack of vigor, reduced productivity, and early senescence of leaves. Air pollutants such as NO_2, O_3, PAN and fluoride can also cause serious injuries to plants. Many fruit trees and vegetables are particularly sensitive to these pollutants.

Assessment of the immediate and long-term economic effects of air pollution on agriculture is difficult because of the many variables involved. However, available information indicates that the cost due to decreased crop yields is staggering. For instance, the 1986 estimated losses to producers caused by O_3 alone were \$1 billion to \$5 billion.[1] The estimated cost of damage caused by acid rain to 32 major crops in the U.S. was \$50 billion.

Injuries to plants by air pollution are often manifested by such symptoms as chlorosis and necrosis. Chlorosis is the fading of natural green color, or yellowing, of plant leaves, and is due to the destruction of chlorophyll or interference with chlorophyll biosynthesis. Necrosis refers to localized or general death of plant tissue and is often characterized by brownish or black discoloration.

3.5 INTOXICATION OF ANIMALS

Many published reports reveal adverse effects in animals that have been exposed to gaseous and particulate forms of air pollutants emitted from industrial facilities. Examples of these facilities include phosphate fertilizer manufacturing plants, aluminum manufacturing plants, iron and other types of smelters, and coal-burning power plants. As is widely known, a large number of air pollutants are emitted from these industrial sources. Animals residing in areas adjacent to these industrial sources are exposed to the pollutants emitted from these sources, resulting in injuries. This is explored further in subsequent chapters.

Similarly, reports of the injuries of fish and wildlife caused by water pollution also abound. Many diseased sea mammals have been washed ashore in different parts of the world in recent years, apparently due to damaged immune systems subsequent to exposure to waterborne toxicants. In the U.S., it is estimated that more than one million waterfowls are killed every year following the ingestion of spent lead pellets left after hunting.

A new type of environmental disease has appeared recently and attracted the attention of many scientists. Beginning in about 1991, biologists noted dramatic declines in amphibian populations and increases in deformities in frogs, with no apparent causes, in remote, high-altitude areas of western U.S., Puerto Rico, Costa Rica, Panama, Colombia, and Australia. The declines

represented a sharp departure from previous years, when amphibian populations had crashed only from habitat destruction or the introduction of exotic predator species. Scientists fear that many species of amphibians that have been around for 350 million years will not survive the 21st century. They view these population losses as an indication that there may be something seriously wrong with the environment. Some scientists suspect that infections, and the effects of synthetic organic compounds (such as pesticides), metallic contaminants, acid precipitation, UV radiation, and increased temperatures may be responsible for the phenomenon. So far, however, there is no conclusive evidence that any of these is responsible for the mysterious declines. Some scientists believe that several factors may be acting synergistically to produce the rapid die-offs.[2]

3.6 INJURIES TO HUMANS

Many individuals in numerous countries have suffered injuries resulting from exposure to high levels of airborne or waterborne pollutants. Exposure to high levels of air pollutants results in various physiological changes, leading to health problems. Air pollutants, such as SO_2, O_3 and other oxidants, and particulate matter, have been regarded as being responsible, solely or in combination, for causing coughing, degeneration of the lining of the throat, pulmonary disease, and heart failure. Some of the injuries result in permanent disability, while others are fatal. Historically, such human injuries occurred only in certain occupations, but in recent years, injuries or deaths have occurred as results of non-occupation-related factors.

Studies show that over the past two decades there has been a startling rise in the prevalence of asthma among children and young adults. This trend persists, mostly in affluent countries.[3] In many of the countries where asthma is common, its prevalence has jumped nearly 50% in 10 years. Rates of hospitalization for asthma are also rising in these countries. For example, asthma mortality among persons of the age group 5 to 34 years rose more than 40% between the mid-1970s and mid-1980s in most countries studied.[4] Although the reason for this trend is not known, many scientists consider it to be associated with environmental factors.

Individuals exposed to toxicants may suffer from various signs and symptoms without knowing the cause at the time of exposure. Furthermore, symptoms may not be manifested immediately following exposure. With cancer, it often takes 15 years or more for symptoms to appear. For example, many of New York's shipyard workers who developed diseases after exposure during the 1940s to asbestos were not diagnosed until 15 to 30 years later. Other examples include Minamata disease and itai-itai-byo, described in Chapter 2. A further example is "yu-sho" or "oil disease," which occurred in Japan as a result of consuming rice oil that was highly contaminated by polychlorinated biphenyls (PCBs).

Human exposure to pesticides can occur directly, especially for agricultural workers and their families. Individuals residing in areas near farms where pesticides are heavily applied may also be directly exposed. Indirect exposure can also occur, e.g., when pesticide residues on food or contaminated fish are ingested. Some synthetic organic pesticides are slow to degrade and persist in the environment for years. Accumulation of various types of pesticides in human tissues can therefore occur and result in health problems.

It is clear that an enormous effort has been made in the U.S. by government, industries, and the public in an attempt to reduce environmental pollution. Such effort has led to a number of encouraging results. According to the U.S. Environmental Protection Agency's 1994 annual assessment of urban air pollution, air quality in the U.S. was improving; however 43 metropolitan regions, home to nearly 100 million Americans, had O_3 levels at more than 0.12 ppm, exceeding federal health standards. In the Los Angeles Basin, in particular, the pollution is so bad that it was given a deadline of 2010 to meet the federal standards.

3.7 ACUTE AND CHRONIC EFFECTS

3.7.1 Introduction

In studying the health effects of toxicants on living organisms, researchers often identify effects as acute or chronic. An acute effect refers to that manifested by severe injuries or even death of an organism, and is characterized by exposure to high concentrations of a toxicant or toxicants for a short period of time. A chronic effect is characterized by a long-term or recurrent exposure to relatively low concentrations of toxicants. Signs and symptoms vary depending on the types of toxicants, their concentrations, and species of exposed organisms.

3.7.2 Acute Effects

A number of acute pollution episodes have occurred in different parts of the world since 1930. A brief review of several major ones follows, and readers are referred to detailed reviews published elsewhere.[5]

3.7.2.1 Meuse Valley, Belgium, 1930

This episode occurred on December 1, 1930 in Meuse Valley, Belgium, where a large number of industrial plants were located. A thermal inversion caused pollutants, such as SO_2, sulfuric acid mist, and particulates, emitted from these plants, to be trapped in the valley. Many people became ill with respiratory discomforts. Reported casualties include 60 human deaths and some deaths in cattle.

3.7.2.2 Donora, Pennsylvania, U.S., 1948

This episode took place on October 26, 1948, and was also due to thermal inversion and foggy weather, which affected a wide area. Many industries, including a large steel mill, a zinc-production plant, and a sulfuric-acid plant, were located in this small industrial city. Nearly half of the population of 14,000 became ill, with coughing being the most prevalent symptom. High levels of SO_2 and particulate matter were the suspected cause of the suffering. This episode resulted in 20 human deaths.

3.7.2.3 Poza Rica, Mexico, 1950

The incident that occurred in the city of Poza Rica, Mexico, in the early morning of November 24, 1950, was caused by the accidental release of H_2S from a natural gas plant. At the time of the accident, most of the nearby residents were still in bed or had just gotten up. Many were quickly affected with symptoms of respiratory distress and central nervous system damage. Twenty-two people died and more than 300 were hospitalized.

3.7.2.4 London, England, 1952

This is the most widely known air pollution episode. It occurred during December 5 through 8, 1952, and was the result of fog and thermal inversion. Many people suffered from shortness of breath. Cyanosis, some fever, and excess fluid in the lungs were reported in many patients. High levels of SO_2, fluoride, and smoke were recorded in the air. According to municipal statistics, approximately 4000 excess deaths occurred. The figure obtained was the difference between the average number of deaths for the same period between 1947 and 1951 and the number of deaths that occurred during the episode (Figure 3.2). Most of those affected were in the older age groups, and generally had disease of the heart or lungs prior to the pollution episode.

3.7.2.5 New York, U.S., 1953

This episode occurred from November 18 to 22, 1953, as a result of air stagnation and the presence of a high level of SO_2 and led to several thousand excess deaths.

3.7.2.6 Los Angeles, California, U.S., 1954

Unlike those events mentioned above, the cause of this episode was smog formation and the accumulation of high levels of photochemical oxidants, such as O_3 and PAN. Excess deaths totaling 247 per day in the 65 to 70 year age group were among the observed consequences.

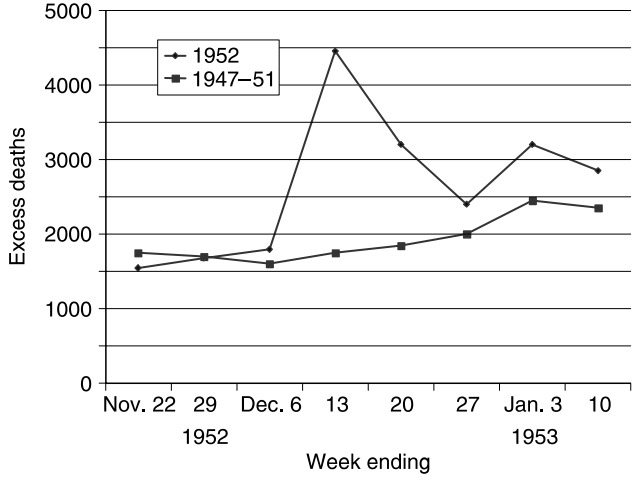

Figure 3.2 Excess deaths in Greater London, England, during the air pollution episode of December 5 to 8, 1952.

3.7.2.7 New Orleans, Louisiana, U.S., 1955

This episode was marked by a sharp increase in the incidence of asthma among the residents of the city. The normal frequency of visits to a local hospital was reported to be an average of 25 per day; but during the episode period it was 200 per day. The suspected cause was dust from flour mills.

3.7.2.8 Worldwide Episode, 1962

This air pollution episode lasted from November 27 to December 10, 1962, and involved the eastern part of the U.S.; London, England; Rotterdam, The Netherlands; Osaka, Japan; Frankfurt, Germany; Paris, France; and Prague, Czechoslovakia. Patients in the U.S. suffered upper respiratory symptoms. There were 700 excess deaths in London, and 60 in Osaka.

3.7.2.9 Tokyo, Japan, 1970

This episode occurred in Tokyo, Japan, on July 18, 1970, and was due to high levels of oxidants and SO_2 in the atmosphere. More than 6000 people complained of severe eye irritation and sore throat. Figure 3.3 shows a smoggy day in Tokyo in 1972, with Tokyo Tower barely visible. (Much improvement in Tokyo's air quality has since been made. Many visitors are impressed with the generally favorable air quality, considering that the city's population is more than 15 million.)

Figure 3.3 Smog in Tokyo in 1972.

3.7.2.10 Bhopal, India, 1984

The worst industrial accident in history occurred in the city of Bhopal, India (Figure 3.4) on the morning of December 3, 1984. Forty tons of the highly toxic gas methyl isocyanate (MIC) ($CH_3-N=C=O$) leaked from a pesticide plant located in Bhopal and diffused into densely populated adjacent neighborhoods. At least 4000 people were killed, and more than 150,000 injured. It was observed that the lung was the main target organ of MIC. A hospital report released three days after the exposure showed the occurrence of interstitial edema, alveolar and interstitial edema, and emphysema among the victims treated.[6] The large number of deaths and injuries (resulting in many permanently disabled), made the accident the greatest acute chemical disaster ever.[7]

3.7.2.11 Chernobyl, USSR, 1986

By far the gravest disaster in the history of commercial atomic power occurred on April 26, 1986, at Chernobyl in Ukraine (Figure 3.5), then a state of the Soviet Union. The No. 4 reactor of the Chernobyl nuclear power station partly melted down and exploded, killing 32 people in the immediate area and causing 237 cases of acute radiation sickness.[8] The explosion sent a devastating cloud of radiation across a wide swath of Europe. Radioactive forms of iodine, cesium, strontium, and plutonium were released into the atmosphere and deposited throughout the northern hemisphere. The 30 km zone surrounding the station, from which 115,000 people were evacuated, received especially high exposure: for the people from this zone the risk of spontaneous leukemia was

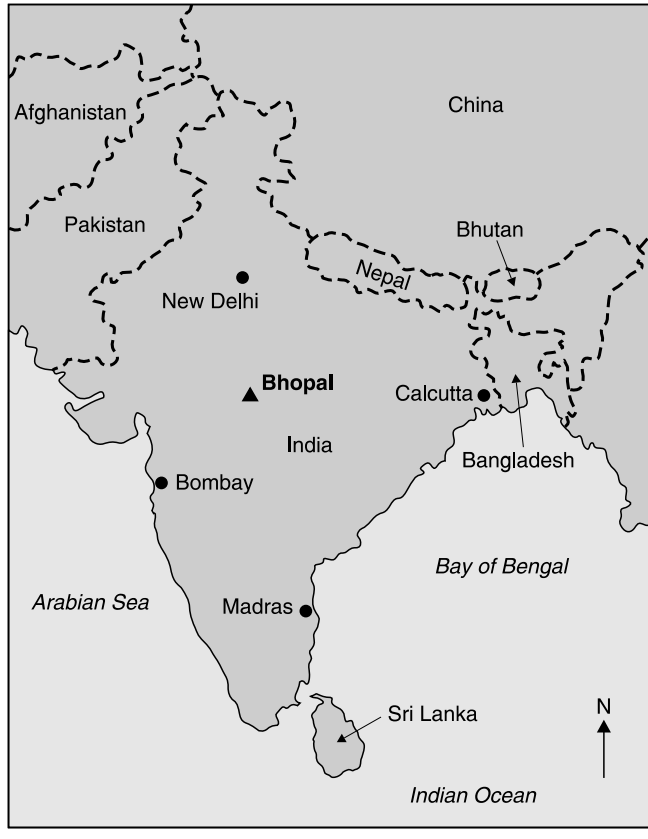

Figure 3.4 Location of Bhopal, India.

estimated to be double for the next decade, and some genetic disorders may appear in individuals who were exposed *in utero*. The total radioactivity of the material released from the reactor was estimated to be 200 times that of the combined releases from the atomic bombs dropped on Hiroshima and Nagasaki, according to a 1995 WHO report.

The accident exposed millions of people, notably in Belarus, Russia, and the Ukraine, to varying doses of radiation. According to the Organization for Economic Cooperation and Development and the Nuclear Energy Agency, 20 radionuclides were released into the atmosphere. They included iodine-131 with a half-life of 8 days; cesium-134 and cesium-137 with half-lives of 2 days and 30 years, respectively; and several plutonium isotopes with half-lives ranging from 13 to 24,000 years. Subsequent studies indicated a dramatic increase in the incidence of thyroid cancer in children, mainly in Belarus and the Ukraine, but also to a lesser extent in Russia.[9]

Figure 3.5 Location of Chernobyl, Ukraine.

3.7.2.12 Prince William Sound, Alaska, U.S., 1989

Crude oil from the North Slope oilfields in Alaska is carried by pipeline to the port of Valdez and then shipped by tanker to the west coast of the U.S. On March 24, 1989, a huge tanker, named Exxon Valdez, went off course in a 16 km-wide channel in Prince William Sound, near Valdez, a harbor town of 4200. The tanker struck a reef, causing the worst ever oil spill in U.S. waters. Eleven million gallons of crude oil escaped, and coated more than 2000 km of shoreline and killing an estimated 250,000 seabirds, 2800 seaotters, 300 harbor seals, 250 bald eagles, as many as 22 killer whales,[10] and countless other marine mammals and fish. The spill also injured an unknown number of salmon and herring eggs and larvae.

While many scientists believed that affected areas had recovered within a decade after the disaster, a new study by Short et al. [11] at the Alaska Fisheries Science Center, NOAA, made an alarming observation. According to the study, oil was found on 78 of 91 beaches randomly selected. The cumulative area of beach contaminated by surface or subsurface oil was estimated at 11.3 ha, and the mass of remaining subsurface oil was about 0.2% of the original oil. The results of their study suggest that the toxicity stemming from the oil, primarily from polyaromatic hydrocarbons (PAHs), continues to affect the recovery of some sea animals in places where the oil is most persistent.[11]

3.7.2.13 Gas Well Accident, Gaoqiao, China, 2003

On December 23, 2003, a leak occurred from a well in a natural-gas field at Gaoqiao, a town in southwest China. The leak spewed out toxic fumes, containing H_2S, which killed at least 191 people and forced 31,000 people living

within three miles of the gas field to flee their homes. The cause of the disaster reportedly involved a drilling mishap, which broke open a gas well.

3.7.3 Chronic Effects

Chronic intoxication is more common than acute episodes of poisoning. Numerous reports have been published relating chronic effects of both air and water pollution on living systems. Long-term exposure to relatively low concentrations of air pollutants, such as SO_2, smoke, and heavy metals (e.g., lead, cadmium, and mercury), may eventually lead to injuries in plants, animals, or humans. The Minamata Bay incident and itai-itai-byo mentioned above are examples of chronic effects related to water pollution. More detailed information is provided in later chapters.

In plants, chronic effects are manifested as impaired growth and development, decreased respiration, chlorosis, necrosis, and other symptoms. Similarly, chronic effects in animals are reflected in retarded growth, increased susceptibility to other environmental stresses, and a variety of adverse health effects, including shorter life spans.

In humans, the health effects of air pollution exposure may occur over a long period of time. A prolonged exposure to air pollutants such as NO_2 and O_3, for instance, may lead to chronic bronchitis and emphysema. In the U. K., the combination of SO_2 and smoke pollution is thought to have synergistic effects with cigarette smoking, causing degenerative diseases.

Results from occupational studies strongly suggest a close association between air pollution exposure and respiratory cancer. For example, inhalation of toxic materials, such as arsenic, asbestos, chromium, soot, mustard gas, and radon, under occupational conditions has been related to lung cancer.[12] In an effort to assess the association between air pollution and daily outpatient hospital visits, Xu et al.[13] collected data on nonsurgery outpatient visits at a community-based hospital in Beijing, China, and on atmospheric SO_2 and total suspended particle (TSP) levels. Analysis of the data showed increases of 20% and 17% in nonsurgery outpatient visits to the hospital, in association with increases in SO_2 and TSP levels, respectively. These observations led to the conclusion that the existing air-pollution levels in Beijing are associated with adverse health effects.

Similar observations were made in Hong Kong. Researchers studied the levels of SO_2, NO_2, O_3, and atmospheric particulate matter less than 10 μm in diameter (PM_{10}) and found a significant association with daily hospital admissions for cardiovascular and respiratory diseases, both combined and separately.[14] Furthermore, the effects of the pollutants on circulatory and respiratory diseases were stronger for older age groups, with significant excess of 5 to 10% in those aged 65 or over. Both NO_2 and O_3 were strongly associated with hospital deaths from cardiovascular and respiratory diseases.[15]

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3.9 REVIEW QUESTIONS

1. What are the differences between acute and chronic injuries?
2. What is chlorosis? How does it occur?
3. What is meant by "excess deaths"?
4. Most of the victims of air pollution episodes are older people or those with prior illnesses. What are the possible reasons for this?
5. What are the differences between a primary pollutant and a secondary pollutant?
6. How is air pollution associated with hospital admissions?
7. Explain the following episodes:
   a) London, U.K., 1952
   b) Bhopal, India, 1984
   c) Chernobyl, USSR, 1986
   d) Alaska, U.S., 1989

Chapter 4

Toxic Action of Pollutants

4.1 INTRODUCTION

When present at a sufficiently high concentration, a pollutant can elicit adverse effects on the living processes of an organism. To exert damage to an exposed organism, a pollutant must first enter the host and reach its target site. A complex pathway exists between the time of initial toxicant exposure and the manifestation of damage by the organism. This chapter discusses general ways in which environmental pollutants exert their actions on plants, animals, and humans.

4.2 PLANTS

4.2.1 Sources of Pollution

For the most part, environmental pollution is an anthropogenic (human-made) problem. As mentioned previously, the most important source of atmospheric pollution in the U.S. is motor vehicles. Other major sources include industrial activities, power generation, space heating, and refuse burning. The composition of pollutants from different sources differs markedly, with industry emitting the most diverse range of pollutants. While carbon monoxide (CO) is the major component of pollution by motor vehicles, sulfur oxides (SO_x) are primary pollutants of industry, power generation, and space heating. In some large cities, such as Los Angeles, accumulation of ozone (O_3), peroxyacyl nitrate (PAN), and other photochemical oxidants constitute the major atmospheric pollution problem.

4.2.2 Pollutant Uptake

Terrestrial plants may be exposed to environmental pollutants in two main ways. One is exposure of forage to air pollutants, another is uptake of toxicants by roots growing in contaminated soils. Vegetation growing near industrial facilities, such as smelters, aluminum refineries, and coal-burning power plants, may absorb airborne pollutants through the leaves and become injured. The pollutants may be in gaseous form, such as sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and hydrofluoric acid (HF), or in particulate form, such as the oxides or salts of metals contained in fly ash (Figure 4.1).

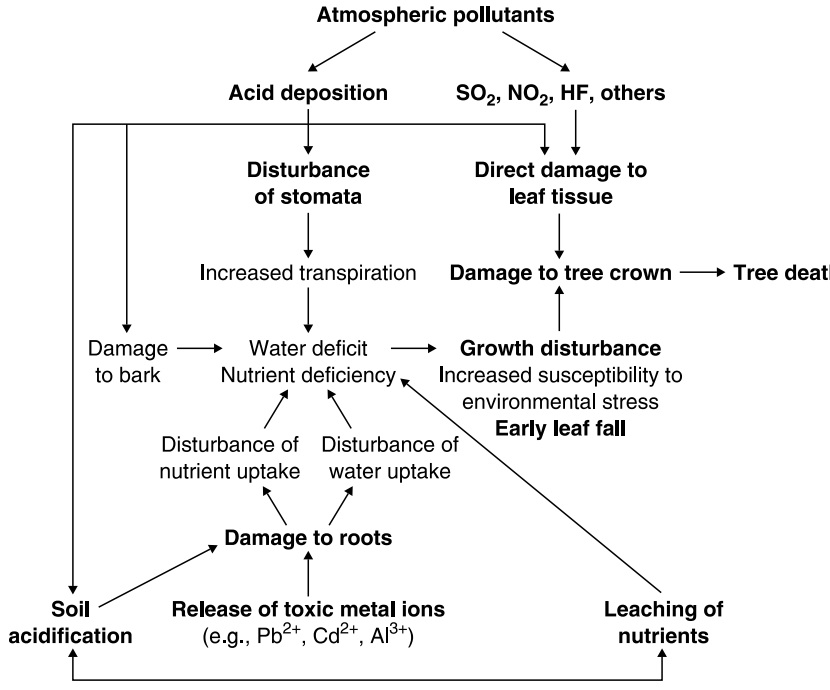

Figure 4.1 Mechanisms of tree damage by air pollutants.

To examine the effect of any airborne pollutants on vegetation, it is crucial to understand the uptake of the pollutants by the plant. While the atmospheric concentration of a pollutant is an essential factor, the actual amount that enters the plant is more important. The conductance through the stomata, which regulate the passage of ambient air into the cells, is especially critical. The extent of uptake depends on the chemical and physical properties of the pollutant along the gas-to-liquid diffusion pathway. The flow of a pollutant may be restricted by the leaf's physical structure (Figure 4.2) or by scavenging chemical reactions occurring within the leaf. Leaf orientation and morphology, including epidermal characteristics, and air movement across the leaf are important determinants affecting the initial flux of gases to the leaf surface.

Stomatal resistance is a very important factor affecting pollutant uptake. The resistance is determined by stomatal size and number, the size of the stomatal aperture, and other anatomical characteristics.[1] Stomatal opening is extremely important: little or no uptake may occur when the stomata are closed. It is regulated by light, humidity, temperature, internal carbon dioxide (CO_2) content, water and nutrient availability to the plant, and potassium (K^+) ions transported into the guard cells.[2]

Exposure of roots to toxicants in contaminated soils is another important process whereby toxicant uptake by plants occurs. For example, vegetation growing in soils of contaminated sites, such as waste sites and areas that have

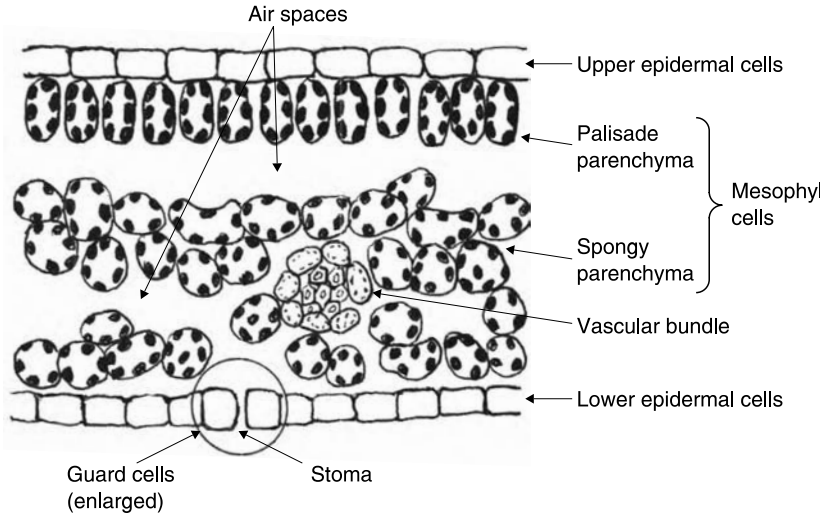

Figure 4.2 Cross section of a leaf, showing the air spaces which serve as passages for pollutants.

received application of contaminated sewage sludge, can absorb toxicants through the roots. In the contaminated sites, high levels of heavy metals, such as lead (Pb) and cadmium (Cd), often occur. Metallic ions are more readily released, and thus more readily absorbed, when the soil is acidified by acid deposition (Figure 4.1).

4.2.3 Transport

Following uptake, a toxicant may undergo mixing with the surrounding medium of the plant, and then be transported to various organs and tissues. Mixing involves the microscopic movement of molecules and is accompanied by compensation of concentration differences. Generally, transport of chemicals in plants occurs passively by diffusion and flux. Diffusion refers to movement across phase boundaries, from a high-concentration compartment to a low-concentration compartment. Flux, on the other hand, is due to the horizontal movement of the medium.

4.2.4 Plant Injury

Besides destroying and killing plants, air pollutants can induce adverse effects on plants in various ways. As noted previously, pollution injury is commonly divided into acute and chronic injury. In plants, an acute injury occurs following absorption of sufficient amounts of toxic gas or other forms of toxicants to cause destruction of tissues. The destruction is often manifested by collapsed leaf margins or other areas, exhibiting an initial water-soaked appearance. Subsequently, the leaf becomes dry and bleaches to an ivory

color or become brown or brownish red. By contrast, a chronic injury may be caused by uptake of sublethal amounts of toxicants over a long period. Chronic injury is manifested by yellowing of leaves that may progress slowly through stages of bleaching until most of the chlorophyll and carotenoids are destroyed.

To cause leaf injury, an air pollutant needs to pass through the stomata of the epidermal tissue, as the epidermis (Figure 4.2) is the first target for the pollutant. In passing into the intercellular spaces, the pollutant may dissolve in the surface water of the leaf cells, affecting cellular pH. A pollutant may not remain in its original form as it passes into solution. Rather, it may be converted into a form that is more reactive and toxic than the original substance. The formation of reactive free radicals following the initial reaction in the cell is an example. The pollutant, either in its original form or in an altered form, may then react with specific cellular constituents, such as cytoplasmic membrane or membranes of the organelles, or with various substances, including enzymes, coenzymes or cofactors, and substrates. The pollutant may then adversely affect cellular metabolism, resulting in plant injury.[3]

An example of a gaseous air pollutant widely known for its damaging effects on plants is SO_2. Once absorbed into the leaf, SO_2 can induce injuries to the ultrastructure of various organelles, including chloroplasts and mitochondria, which in turn can lead to disruption of photosynthesis or cellular energy metabolism. Similarly, histochemical studies of fluoride-induced injury have indicated that the damage to leaves first occurs in the spongy mesophyll and lower epidermis, followed by distortion or disruption of chloroplast in the palisade cells.[4]

As a pollutant moves from the substomatal regions to the cellular sites of perturbation, it may encounter various obstacles along the pathway. Scavenging reactions between endogenous substances and the pollutant may occur, and the result may affect pollutant toxicity. For example, ascorbate, which occurs widely in plant cells, may react with or neutralize a particular pollutant or a secondary substance formed as the pollutant is metabolized. Conversely, an oxidant such as O_3 may react with membrane material and induce peroxidation of the lipid components. This is followed by the formation of various forms of toxic substances, such as aldehydes, ketones, and free radicals.[5,6] The free radicals, in turn, may attack cellular components, such as proteins, lipids, and nucleic acids, which can lead to tissue damage. Endogenous antioxidants, such as ascorbic acid mentioned above, may react with free radicals and alter their toxicity.

Cellular enzyme inhibition is often observed when leaves are exposed to atmospheric pollutants. The inhibition occurs even before the leaf injuries become apparent. For instance, fluoride (F), widely known as a metabolic inhibitor, can inhibit a large number of enzymes. Fluoride-dependent enzyme inhibition is often attributable to reaction of F^- with certain metallic cofactors such as Cu^{2+} or Mg^{2+} in an enzyme system. Heavy metals, such as Pb and Cd, may also inhibit enzymes that contain a sulfhydryl (−SH) group at the active

site. Alternatively, SO_2 may oxidize and break apart the sulfur bonds in critical enzymes of the membrane, disrupting cellular function.

As noted previously, soil acidification increases release of toxic metal ions, such as Pb^{2+} and Cd^{2+} ions. These metal ions may directly damage roots by disrupting water and nutrient uptake, resulting in water deficit or nutrient deficiency. Soil acidification can also cause leaching of nutrients, leading to nutrient deficiency and growth disturbance (Figure 4.1). A plant becomes unhealthy as a result of one or more of the disturbances mentioned above. Even before visible symptoms are discernable, an exposed plant may be weakened and its growth impaired. In time, visible symptoms, such as chlorosis or necrosis, may appear, followed by death.

4.3 MAMMALIAN ORGANISMS

4.3.1 Exposure

An environmental pollutant may enter an animal or human through a variety of pathways. Figure 4.3 shows the general pathways that pollutants follow in mammalian organisms. As mentioned earlier, exposure of a host organism to a pollutant constitutes the initial step in the manifestation of toxicity. A mammalian organism may be exposed to pollutants through inhalation, dermal contact, eye contact, or ingestion.

4.3.2 Uptake

The immediate and long-term effects of a pollutant are directly related to its mode of entry. The portals of entry for an atmospheric pollutant are the skin, eyes, lungs, and gastrointestinal tract. The hair follicles, sweat glands, and open wounds are the possible entry sites where uptake from the skin may occur. Both gaseous and particulate forms of air pollutants can be taken up through the lungs. Uptake of toxicants by gastrointestinal tract may occur when consumed foods or beverages are contaminated by air pollutants, such as Pb, Cd, or sprayed pesticides.

For a pollutant to be taken up into the body and finally carried to a cell, it must pass through several layers of biological membranes. These include not only the peripheral tissue membranes, but also the capillary and cell membranes. Therefore, the nature of the membranes and the chemical and physical properties (e.g., lipophilicity) of the toxicant in question are important factors affecting uptake. The mechanisms by which chemical agents pass through the membrane include:

- filtration through spaces or pores in membranes
- passive diffusion through the spaces or pores, or by dissolving in the lipid material of the membrane

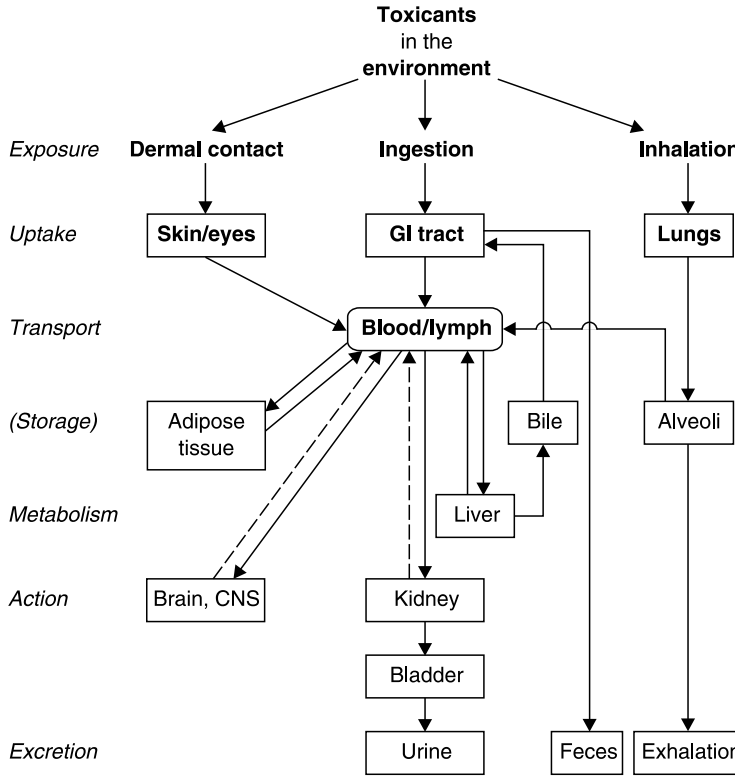

Figure 4.3 Processes of poisoning in animals and humans.

- facilitated transport, where a specialized protein molecule, called a *carrier*, carries a water-soluble substance across the membrane
- active transport, which requires both a carrier and energy

Of the four mechanisms, active transport is the only one where a toxicant can move against a concentration gradient, i.e., move from a low-concentration compartment to a high-concentration compartment (Table 4.1). This accounts for the need for energy expenditure.

4.3.3 Transport

Immediately after absorption, a toxicant may be bound to a blood protein (such as lipoprotein), forming a complex, or it may exist in a free form. Rapid transport throughout the body follows. Transport of a toxicant may occur through the bloodstream or lymphatic system. The toxicant may then be distributed to various body tissues, including those of storage depots and sites of metabolism.

Table 4.1 Four Basic Types of Absorption Processes

Process	Energy needed	Carrier	Concentration gradient
Passive	No	No	High→low
Facilitated	No	Yes	High→low
Active	Yes	Yes	High→low Low→high
Phagocytosis/pinocytosis[a]	Yes	No	NA

Note: NA = not applicable.
[a]Phagocytosis is involved in invagination of solid particles, whereas pinocytosis is involved in uptake of liquids.

4.3.4 Storage

A toxicant may be stored in the liver, lungs, kidneys, bone, or adipose tissue. These storage *depots* may or may not be the sites of toxic action. A toxicant may be stored in a depot temporarily and then released and translocated again. Similarly, a toxicant or its metabolite may be transported to a storage site and remain there for a long period of time, even permanently. Excretion of the toxicant following temporary storage may also occur.

4.3.5 Metabolism

The metabolism of toxicants may occur at the portals of entry, or in such organs as the skin, lungs, liver, kidney, and gastrointestinal tract. The liver plays a central role in the metabolism of environmental toxicants (xenobiotics). The metabolism of xenobiotics is often referred to as biotransformation. The liver contains a rich supply of nonspecific enzymes, enabling it to metabolize a broad spectrum of organic compounds.

Biotransformation reactions are classified into two phases, Phase I and Phase II. Phase I reactions are further divided into three main categories, oxidation, reduction, and hydrolysis. These reactions are characterized by the introduction of a reactive polar group into the xenobiotic, forming a primary metabolite. In contrast, Phase II reactions involve conjugation reactions in which the primary metabolite combines with an endogenous substance, such as certain amino acids or glutathione (GSH), to form a complex secondary metabolite. The resultant secondary metabolite is more water-soluble, and therefore more readily excreted, than the original toxicant.

While many xenobiotics are detoxified as a result of these reactions, others may be converted to more active and more toxic compounds. Biotransformation will be discussed in more detail in Chapter 5.

4.3.6 Excretion

The final step in the pathway of a toxicant is its excretion from the body. Excretion may occur through the lungs, kidneys, or gastrointestinal tract. A toxicant may be excreted in its original form or as its metabolites, depending

on its chemical property. Excretion is the most permanent means whereby toxic substances are removed from the body.

4.4 MECHANISM OF ACTION

The toxic action of pollutants involves either compounds with intrinsic toxicity or activated metabolites. These interact with cellular components at specific sites of action to cause toxic effects, which may occur anywhere in the body. The consequences of such action may be reflected in changes in physiological and biochemical processes within the exposed organism. These changes may be manifested in different ways, including impaired central nervous system (CNS) function and oxidative metabolism, injury to the reproductive system, or altered DNA leading to carcinogenesis.

The duration of toxic action depends on the characteristics of the toxicant and the physiological or biochemical functioning of the host organism. Generally, the toxic action of a xenobiotic may be terminated by storage, biotransformation, or excretion.

The mechanisms involved in xenobiotic-induced toxicity are complex and much remains to be elucidated. The ways in which xenobiotics can induce adverse effects in living organisms include:

- disruption or destruction of cellular structure
- direct chemical combination with a cell constituent
- inhibition of enzymes
- initiation of a secondary action
- free-radical-mediated reactions
- disruption of reproductive function

These mechanisms are examined in the following sections.

4.4.1 Disruption or Destruction of Cellular Structure

A toxicant may induce an injurious effect on plant or animal tissues by disrupting or destroying the cellular structure. As mentioned previously, atmospheric pollutants, such as SO_2, NO_2, and O_3, are phytotoxic – they can cause plant injuries. Sensitive plants exposed to any of these pollutants at sufficiently high concentrations may exhibit structural damage when their tissue cells are destroyed. Studies show that low concentrations of SO_2 can injure epidermal and guard cells, leading to enhanced stomatal conductance and greater entry of the pollutant into leaves.[1] Similarly, after entry into the substomatal cavity of the plant leaf, O_3, or the free radicals produced from it, may react with protein or lipid membrane components, disrupting the cellular structure of the leaf.[3,5]

In animals and humans, inhalation of sufficient quantities of NO_2 and sulfuric acid mists can damage surface layers of the respiratory system.

Similarly, high levels of O_3 can induce peroxidation of the polyunsaturated fatty acids in the lipid portion of membranes, resulting in disruption of membrane structure.[6]

4.4.2 Chemical Combination with a Cell Constituent

A pollutant may combine with a cell constituent, forming a complex and disrupting cellular metabolism. For example, CO is widely known for its ability to bind to hemoglobin (Hb). After its inhalation and diffusion into the blood, CO readily reacts with Hb to form carboxyhemoglobin (COHb):

$$CO + Hb \rightarrow COHb \tag{4.1}$$

The presence of a large amount of COHb in the blood disrupts the vital system for exchange of CO_2 and O_2 between the blood and the lungs and other body tissues. Interference with the functioning of hemoglobin by COHb accumulation is detrimental to health and can lead to death.

A number of toxicants or their metabolites are capable of binding to DNA to form DNA adducts. Formation of such adducts results in structural changes in DNA, leading to carcinogenesis. For instance, benzo[a]pyrene, one of the many polycyclic aromatic hydrocarbons (PAHs), may be converted to its epoxide form in the body. The resultant epoxide can in turn react with guanine on a DNA molecule to form a guanine adduct. Another example is found with alkylating agents. These chemicals are metabolized to reactive alkyl radicals, which can also induce adduct formation. These will be discussed in more detail in Chapter 16.

Certain metallic cations can interact with the anionic phosphate groups of polynucleotides. They can also bind to polynucleotides at various specific molecular sites, particularly purines and thymine. Such metallic cations can, therefore, inhibit DNA replication and RNA synthesis and cause nucleotide mispairing in polynucleotides. An anatomical feature of chronic intoxication of Pb in humans and in various animals is the presence of characteristic intranuclear inclusions in proximal tubular epithelial cells in the kidneys. These inclusions appear to be formed from Pb and soluble proteins.[7] By tying up cellular proteins, Pb can depress or destroy their function.

4.4.3 Effect on Enzymes

The most distinctive feature of reactions that occur in living cells is the participation of enzymes as biological catalysts. Almost all enzymes are proteins with a globular structure, and many of them carry out their catalytic function by relying solely on their structure. Many others require nonprotein components, called *cofactors*. Cofactors may be metal ions or alternatively they may be organic molecules, called *coenzymes*. Metal ions capable of acting as cofactors include K^+, Na^+, Cu^{2+}, Fe^{2+} or Fe^{3+}, Mg^{2+}, Mn^{2+}, Ca^{2+}, and Zn^{2+} ions (Table 4.2). Examples of coenzymes that serve as transient carriers of

Table 4.2 Metallic Ions and Some Enzymes That Require Them

Metallic ion	Enzyme
Ca^{2+}	Lipase, α-amylase
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Catalase, cytochrome oxidase, peroxidase
K^{+}	Pyruvate kinase (also requires Mg^{2+})
Mg^{2+}	Hexokinase, ATPase, enolase
Se	Glutathione peroxidase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, DNA polymerase

specific atoms or functional groups are presented in Table 4.3. Many coenzymes are vitamins or contain vitamins as part of their structure. Usually, a coenzyme is firmly bound to its enzyme protein, and it is difficult to separate the two. Such tightly bound coenzymes are referred to as *prosthetic groups* of the enzyme. The catalytically active complex of protein and prosthetic group is called *holoenzyme*, while the protein without the prosthetic group is called *apoenzyme*, which is catalytically inactive (Reaction 4.2).

$$\underset{\text{(Apoenzyme)}}{\text{Enzyme}} + \text{prosthetic group} \rightarrow \underset{\text{(Holoenzyme)}}{\text{Protein–prosthetic group}} \tag{4.2}$$

Coenzymes are especially important in animal and human nutrition because, as previously mentioned, most are vitamins or are substances produced from vitamins. For example, niacin, after being absorbed into the body, is converted to nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH), important coenzymes in cellular metabolism.

There are several ways in which toxicants can inhibit enzymes, leading to disruption of metabolic pathways. Some examples are given below.

4.4.3.1 Enzyme Inhibition by Inactivation of Cofactor

As mentioned above, some cofactors in an enzyme system are metallic ions, which provide electrophilic centers in the active site, facilitating catalytic reactions. For instance, fluoride (F) has been shown to inhibit α-amylase, an

Table 4.3 Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Entity transferred
Coenzyme A	Acyl group
Flavin adenine dinucleotide	Hydrogen atoms
Nicotinamide adenine dinucleotide	Hydride ion (H^{-})
Thiamin pyrophosphate	Aldehydes
Biotin	CO_2

enzyme responsible for the breakdown of starch into maltose and eventually glucose – the released glucose is then used as energy source. α-amylase is known to require Ca^{2+} for its stability as well as catalytic action.[8,9] In the presence of F^- ions, α-amylase activity is depressed.[10,11] The mechanism involved in the inhibition appears to involve removal of the Ca^{2+} cofactor by F^- ions. Evidence supporting this observation was obtained when a crude enzyme extract from mung bean seedlings exposed to 5 m*M* NaF for 3 days was tested for activity. When the enzyme extract was incubated with $CaCl_2$ for 30, 60, and 90 minutes, α-amylase activity was much higher than the activity shown by the control assay mixture without added $CaCl_2$ (Figure 4.4).[11]

Fluoride is also known to inhibit enolase, an enzyme involved in glycolysis. Enolase requires Mg^{2+} as a cofactor (see Chapter 10, Figure 10.7). The F-induced inhibition of the enzyme is more marked in the presence of phosphate. It is therefore generally assumed that the mechanism involved in inhibition is by inactivation of the cofactor Mg resulting from formation of magnesium-fluorophosphate.

4.4.3.2 Enzyme Inhibition by Competition with Cofactor

Many enzymes carry out their catalytic function depending solely on their protein structure. Many others require nonprotein *cofactors* for their functioning. Cofactors may be metal ions or organic molecules referred to as *coenzymes*. Table 4.2 shows several metal ions and some enzymes that require them, while examples of several coenzymes and representative enzymes using the coenzymes are presented in Table 4.3. As shown in the Table 4.2, several enzymes require Zn^{2+} ions as a cofactor. Cadmium (Cd^{2+}), which is chemically similar to Zn^{2+}, can inhibit these enzymes by competing with the Zn^{2+}cofactor.

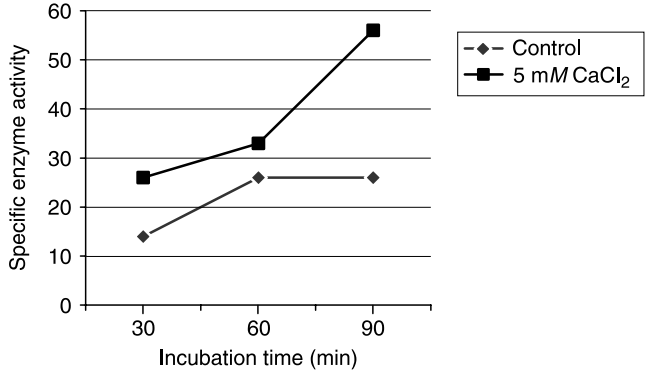

Figure 4.4 Effect of Ca on α-amylase activity in mung bean seedlings exposed to NaF. Enzyme extracts were prepared from seedlings exposed to 5.0 m*M* NaF for 24 hours. Enzyme assay mixture contained Tris-buffer (pH 7.0), 0.2% starch solution, and water (control) or 5 m*M* CaCl2, and the mixture was incubated for a total of 90 minutes. Glucose produced at each incubation period was determined for specific activity determination.)
Source: Yu, M., Shumway, M., and Brockbank, A., *J. Fluorine Chem.*, 41, 95, 1988.

Beryllium (Be) is known to inhibit certain enzymes that require Mg^{2+} for a similar reason.

4.4.3.3 Enzyme Inhibition by Binding to the Active Site

A toxicant may bind to the active site of an enzyme. For instance, a thiol or sulfhydryl (−SH) group on a protein enzyme often is the active site for the catalytic action of the enzyme. A heavy metal, such as Pb, Cd, or Hg, after absorption into the body may attach itself to the −SH group, forming a covalent bond with the sulfur atom (Reaction 4.3). With the active site being blocked, the activity of the enzyme will be depressed or lost.

$$2Enz\text{–SH} + Pb^{2+} \rightarrow Enz\text{–S–Pb–S-}Enz + 2H^{+} \tag{4.3}$$

For example, alanine aminotransferase (the enzyme that catalyzes the transamination of alanine) and δ-aminolevulinate dehydratase (ALAD, a key enzyme in the heme synthetic pathway) both have −SH groups as active sites. Pb strongly inhibits both of these enzymes by the same mechanism.

Another example is the widely known inhibition of acetylcholinesterase (AChE) by chemicals such as organophosphate. Acetylcholinesterase is the enzyme responsible for the breakdown of acetylcholine (ACh), the neurotransmitter in insect and vertebrate nervous systems (Reaction 4.4).

$$\underset{\text{Acetylcholine (ACh)}}{(CH_3)_3N^{+}-CH_2CH_2-O-\overset{\overset{O}{\|}}{C}-CH_3} \xrightarrow{\text{AChE}} \underset{\text{Choline}}{(CH_3)_3N^{+}-CH_2CH_2OH} + \underset{\text{Acetic acid}}{CH_3COOH} \tag{4.4}$$

When AChE is inhibited, ACh will accumulate and keep firing at the nerve endings. As a result, the nerve functioning is interrupted, which may lead to death of the affected organism.

Evidence suggests that the vertebrate AChE contains two binding sites, one of them being serine (an amino acid) with the $–CH_2OH$ residue as the active site. Chemicals such as organophosphate pesticides, which can inactivate AChE, are known to attach to the functional group $–CH_2OH$ in serine on the enzyme molecule by forming a covalent bond (see Section 13.2.2.3).

4.4.3.4 Enzyme Activity Depression by Toxic Metabolite

In this case, enzyme inhibition is not caused by the toxicant itself, but rather by its metabolite. For example, sodium fluoroacetate, known as Rat Poison 1080, is extremely toxic to animals. However, the toxicity is not due to sodium fluoroacetate itself but rather to a metabolic conversion product, fluorocitrate, formed through a reaction commonly known as *lethal synthesis* (Figure 4.5).

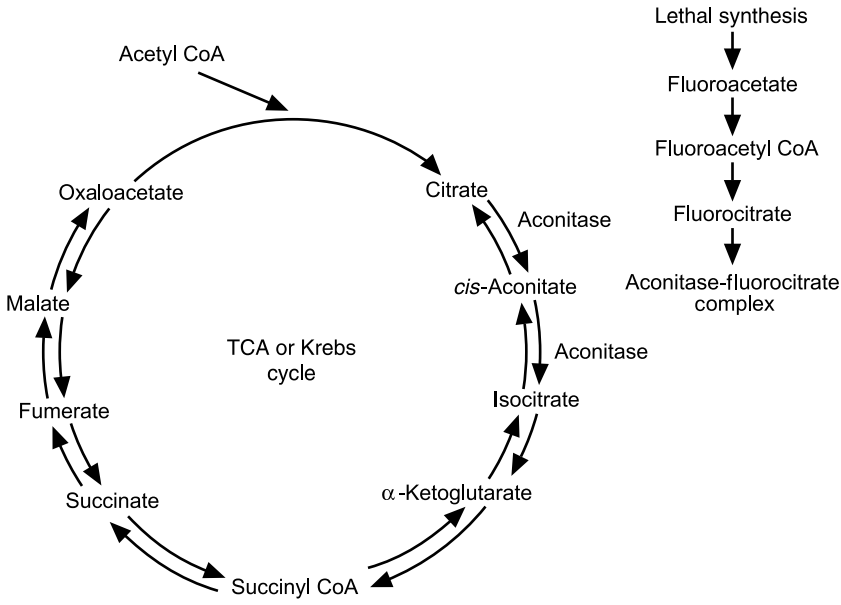

Figure 4.5 Synthesis of fluorocitrate from fluroacetate through lethal synthesis. Inhibition of aconitase shuts down the Krebs cycle.

The resultant fluorocitrate is toxic because it is a potent inhibitor of aconitase, the enzyme that catalyzes the conversion of citrate into *cis*-aconitate and then into isocitrate (in the Krebs cycle). Inhibition of aconitase results in citrate accumulation. The outcome of this inhibition is an impaired Krebs cycle function, which therefore disrupts energy metabolism.

4.4.4 Secondary Action as a Result of the Presence of a Pollutant

The presence of a pollutant in a living system may cause the release of certain substances that are injurious to cells. Several examples are given below.

4.4.4.1 Allergic Response to Pollen

In many individuals, allergic response occurs after inhalation of pollen, leading to common symptoms of hay fever. These symptoms are due to the release of histamine, a substance formed from the amino acid histidine through decarboxylation. Histamine is made and stored in the mast cell and in many other cells of the body. Release of histamine occurs in anaphylaxis, or as a consequence of allergy; it is also triggered by certain drugs and chemicals. Histamine is a powerful vasodilator, capable of causing dilation and increasing blood vessel permeability. Histamine also stimulates pepsin secretion, can reduce the blood pressure and, if severe enough, induce shock. When present in excessive levels, histamine can cause vascular collapse. Antihistamines, such as

diphenylhydramine and antergan, are compounds whose structures are similar to that of histamine and can prevent physiologic changes induced by histamine.

4.4.4.2 Carbon Tetrachloride

The way in which carbon tetrachloride (CCl_4) affects humans is another example. Once taken up into the body, CCl_4 is known to cause a massive discharge of epinephrine from sympathetic nerves, eventually resulting in liver damage. Epinephrine is a potent hormone, involved in many critical biological reactions in animals and humans, including:

- stimulation of glycogenolysis (breakdown of glycogen into glucose) in the liver and muscle: in the liver, the resultant glucose enters blood circulation; in the muscle, the resultant glucose does not enter blood circulation but instead is converted to lactic acid before being transferred back to the liver
- lipolysis (breakdown of fats): involves the breakdown of triacylglycerol into fatty acids and glycerol
- glucagon secretion
- inhibition of glucose uptake by muscle
- insulin secretion

Epinephrine also causes the blood pressure to rise. Like other hormones, epinephrine is rapidly broken down when it finishes its function. The breakdown of epinephrine occurs mainly in the liver. Studies show that in the liver CCl_4 is broken down into reactive free radicals, i.e., $^{\cdot}CCl_3$ and $Cl^{\cdot}$ (Reaction 4.5). It is suggested that the free radicals, in turn, can damage liver by reacting with liver cellular components.

$$CCl_4 \xrightarrow{\text{Cytochrome P450}} {}^{\cdot}CCl_3 + Cl^{\cdot} \tag{4.5}$$

4.4.4.3 Chelation

Chelation is a process wherein atoms of a metal in solution are sequestered by ring-shaped molecules. The ring of atoms, usually with O, N, or S as an electron donor, has the metal as an electron acceptor. The metal is more firmly gripped within this ring than if it were attached to separate molecules. The formation of strain-free stable chelate rings requires at least two atoms that can attach to a metal ion. The iron in a hemoglobin molecule and the magnesium in a chlorophyll molecule are two such examples. Through chelation, some biologically active compounds are absorbed and retained in the body, whereas others may be removed from it.

Some researchers suggest that the toxicity of certain chemicals may be attributed to chelation. For instance, when rabbits were exposed to carbon disulfide (CS_2) at 250 ppm, a rapid outpouring of tissue Zn in urine occurred. The loss of body Zn is primarily due to a chemical reaction of CS_2 with free

amino groups of tissue protein, forming thiocarbamate and thiazolidone, which might form soluble chelate with Zn.[12]

It has been suggested that metal chelation may be one of the mechanisms involved in carcinogenesis. Many carcinogens have, or can be metabolized to, chemical species capable of metal-binding. This in turn may aid the entrance of metals into cells. Once inside the cells, interaction between normal metals and abnormal metals may occur, resulting in alteration of cellular metabolism.

4.4.4.4 Metal shift

The phenomenon called metal shift may account for some of the responses seen in animals that are exposed to certain toxicants. *Metal shift* refers to movement of metals from one organ to another due to the presence of a toxicant, and is among the earliest biological indicators of toxic response. For example, rats exposed to F show an increase in serum Zn content, whereas the levels of Se and Al in the rats' whiskers were decreased.[13] A similar change was observed with rats exposed to O_3. When exposed to O_3 for 4 hours, the rats showed increased levels of Cu, Mo, and Zn in their lungs, while the levels of these metals in the liver were decreased.

4.4.5 Free-Radical-Mediated Reactions

A free radical is any molecule with an odd number of electrons, and can occur as both organic and inorganic molecules. Free radicals are highly reactive and therefore highly unstable and short-lived. For instance, the half-life of lipid peroxyl radical ($ROO^{\cdot}$) is 7 seconds, and that of hydroxyl radical ($HO^{\cdot}$) is 10^{-9} seconds

Free radicals are derived from both natural and anthropogenic sources. They are produced naturally *in vivo* as byproducts from normal metabolism. Some of the examples include superoxide free radical ($O_2^{\cdot -}$) and H_2O_2. Anthropogenic sources of free radicals are found in such situations as when an organism is exposed to ionizing radiation, certain drugs, or various xenobiotics. The free radicals thus produced can cause chain reactions and damage critical cellular constituents, including proteins, lipids, and DNA. In proteins, the consequence of free-radical attacks is manifested by peptide-chain scission and denaturation. With DNA, strand scission or base modification may occur, potentially leading to cell mutation and death. Researchers generally agree that many human diseases, including heart disease and certain types of cancer, are attributable, at least partly, to free-radical-mediated reactions.

As free radicals react with the unsaturated fatty acids and cholesterol, such as those in cellular membranes, they can induce lipid peroxidation. This process, in turn, can become autocatalytic after initiation, leading to the production of lipid peroxide, lipid alcohol, aldehydes and other chemical species.[14] Interaction with other cellular constituents can also occur, thus injuring cells. Obviously, by inducing these reactions, free radicals can damage cell plasma membranes, and those of organelles.

Certain atmospheric pollutants, such as O_3, PAN, and NO_2, can act as free radicals themselves. Extensive studies have been conducted on the nature of O_3-dependent peroxidation of lipid material in both plants and animals. Lipid peroxidation can also occur as a result of free-radical-dependent reactions initiated by other environmental agents. Figure 4.6 shows the mechanism involved in lipid peroxidation. It also shows the initiation of a chain reaction that can occur following the formation of new species of free radical. As a result of peroxidation and subsequent reactions, the nature of lipid material is altered and cellular functions are disrupted.

Studies show that free radicals such as the hydroxyl radical ($OH^{\cdot}$) can cause peroxidation or crosslinking of membrane lipids and intracellular compounds, thus leading to cell aging and death. Although this is part of the normal aging process of cells, the presence of increased oxidative stress is thought to lead to premature cell aging. For example, the potentially harmful reactivity and oxidative potential of iron (Fe) are carefully modulated within living organisms, by the binding of Fe to carrier proteins or by the presence of other molecules with antioxidant properties. When not properly controlled, redox reactions can cause major damage to cellular components, such as fatty acids, proteins, and nucleic acids. Iron catalyzes the Fenton reaction, one of the best-known processes for converting superoxide and hydrogen peroxide to very reactive free radicals (Reaction 4.6 and Reaction 4.7).

$$O_2 + Fe^{+3} \rightarrow O_2^{\cdot} + Fe^{+2} \tag{4.6}$$

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^{-} + OH^{\cdot} \tag{4.7}$$

4.4.6 Endocrine Disruption

Estrogen, a steroid hormone, is produced in both males and females. It is produced in much large quantities in females and, therefore, is considered a female hormone. In both humans and animals, a specific ratio of estrogen to androgens (male hormones) is necessary for sexual differentiation in the developing fetus. If the ratio is perturbed, the offspring may be born with two sets of partially developed sexual organs (intersex), or with a single set that is incompletely or improperly developed.

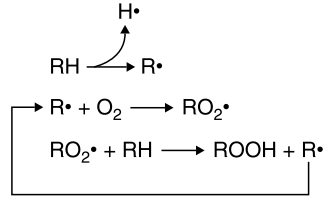

Figure 4.6 Lipid peroxidation and production of lipid free radicals. RH = polyunsaturated fatty acid; $R^{\cdot}$ = lipid (fatty acid) free radical; $ROO^{\cdot}$ = lipid peroxide free radical; ROOH = lipid/organic hydroperoxide.

Estrogenicity is mediated by binding to specific intracellular proteins known as *receptors*. This binding causes a conformational change in the receptor, enabling the estrogen–estrogen receptor complex to bind to specific sites on DNA. Once bound to DNA, the complex alters the expression of estrogen-responsiveness genes. Steroidal estrogens exert their effects through this change in gene expression (Figure 4.7). An exogenous chemical agent can alter the receptor-mediated process by a number of mechanisms. For example, the chemical agent may change the level of endogenous estrogen at a particular site by altering its synthesis, metabolism, distribution, or clearance. Alternatively, the chemical may modify tissue responsiveness to estrogen by changing receptor levels or by acting through a secondary pathway to influence receptor function. Finally, a chemical may attach itself to the estrogen receptor in cells and mimic or block estrogenicity.[15] *Endocrine disrupters* are therefore defined as exogenous chemical agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones.[16]

A particular group of chemicals, called *estrogen mimics*, is able to imitate the action of estrogen. The estrogen mimics are a diverse range of chemicals with no obvious structural similarity. Nevertheless, major characteristics of these chemicals have been elucidated. These chemicals are highly persistent, highly fat-soluble, and have a high potential to accumulate in fat tissue of animals and humans. Some examples of estrogen mimics include DDT, DDE, dieldrin, Kepone, methoxychlor, and polychlorinated biphenyls.[17]

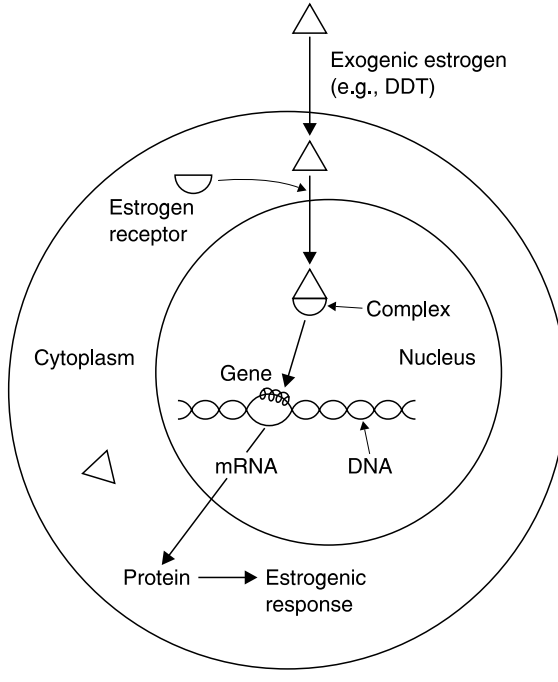

Figure 4.7 Impact of exogenic estrogen, an endocrine disrupter, on gene expression within a cell.

For example, DDT has been shown to cause reproductive failure in western gulls in California.[18] The poor breeding success was characterized by a reduced number of adult males, a highly skewed sex ratio (e.g., female to male ratios of 3.85 on Santa Barbara Island), and female–female pairing of some of the excess females. Researchers suggest that the causes for the observed poor breeding success might include DDT contamination, causing the thinning of eggshells and also abnormal development of the reproductive system in embryos leading to breeding failure in the adult birds.

Research conducted in the past two decades indicate that certain persistent toxicants may be producing adverse effects in wildlife, including birds and mammals, and in humans, by disrupting the endocrine system. Some of the effects include reproductive and developmental abnormalities, increases in certain hormone-related cancers, such as breast, testis, and prostate cancers, and decreases in wildlife populations.

Similarly, a number of other reports have indicated that sperm counts in men worldwide have decreased about 50% since 1940. Over the same period, the incidence of prostate cancer in some countries has doubled, while that of testicular cancer has tripled. There are also indications that birth defects in the male reproductive tract have increased over the past several decades. Furthermore, since 1940, the incidence of female breast cancer has risen in the U.S. and Western Europe. Studies also show that endometriosis (the growth outside the uterus of cells that normally line the uterus), formerly a rare condition, now afflicts five million American women. Women who are afflicted by the disease in their reproductive years frequently suffer infertility.

In the animal world, a study of alligators on Lake Apopka in Florida found that the young were often unable to hatch, and that males that did hatch had abnormally small penises. An active program of research followed the observation, and a large number of reports related to the subject have been published. Many scientists agree that at least part of the reason for the observed conditions may be the introduction into the environment since 1940 of xenobiotics that block or mimic the action of estrogen.

Such chemicals may act on the adult human or animal and cause cancer or endometriosis. The consequences may be even more widespread and devastating when estrogen mimics accumulate in the mother. The estrogen mimics may then be transferred to the egg or fetus, disrupting the hormone balance of the developing offspring and causing reproductive abnormalities or changes that set the stage for cancer in adulthood. (Further discussion of endocrine disruption is presented in Chapter 14.)

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4.6 REVIEW QUESTIONS

1. Which is more injurious to plants/animals exposed to pollutants continuously or intermittently?
2. Explain the relationship between acid rain and plant injury.
3. Why is acidified soil more harmful to plants than non-acidified soil?

4. Explain the way in which Pb may inhibit an enzyme.
5. Explain the way in which fluoride may inhibit an enzyme.
6. What is meant by facilitated transport?
7. What does active transport refer to? What are the characteristics involved in this process?
8. List the three main reactions involved in Phase I reaction.
9. Explain the main feature involved in Phase II reaction.
10. List four endogenous substances that may be involved in conjugation reactions.
11. Explain how a toxicant may directly combine with a cell constituent and cause injury.
12. What is meant by lethal synthesis?
13. List several metallic ions that can act as a cofactor in an enzyme system.
14. Explain how cell membranes may be disrupted by Cd or Pb.
15. Explain how acetylcholinesterase (AChE) may be inhibited.
16. Explain how SO_2 may damage leaf tissues.
17. What is a free radical? How is it produced?
18. Explain how ozone may injure lipid membranes.
19. Explain the process involved in lipid peroxidation.
20. Explain the way in which cellular macromolecules may be affected by free radicals.
21. Briefly describe the process involved in estrogenicity.
22. What is meant by an estrogen mimic?
23. What are the major characteristics of estrogen mimics?
24. Give the names of five chemicals that can act as estrogen mimics.
25. Briefly explain the ways in which environmental chemicals may affect the receptor-mediated process.

Chapter 5

Factors Affecting Xenobiotic Action

5.1 INTRODUCTION

Many factors can affect toxicity of xenobiotics. This chapter examines some of these factors, including physicochemical properties of toxicants, dose or concentration, mode and duration of exposure, environmental factors, interaction, and biological and nutritional factors.

5.2 PHYSICOCHEMICAL PROPERTIES

Physical and chemical characteristics – such as whether a pollutant is solid, liquid, or gas, whether it is soluble in water or in lipid, organic or inorganic material, ionized or non-ionized, etc. – can affect the ultimate toxicity of a pollutant. For instance, a non-ionized substance may be more toxic than an ionized or charged counterpart because the non-ionized species can pass through the membrane more easily than the ionized species and, therefore, is more readily absorbed and able to elicit its toxic action.

5.3 DOSE OR CONCENTRATION

Dose or concentration of any pollutant to which an organism is exposed is often the most important factor affecting its toxicity. Once a pollutant gains entry into a living organism and reaches its target site, it may exhibit an injurious action. For this reason, any factors capable of modifying internal concentrations of the pollutant can alter its toxicity. The effect of the pollutant is therefore a function of its concentration at the locus of its action. A pollutant may either depress or stimulate normal metabolic function. In general, minute amounts of a pollutant may stimulate metabolic function, whereas large doses may impede or destroy its activity. For example, a recent epidemiological study showed that in the area of Kuitan, a city situated in the western part of China, many residents suffer from arsenism, a disease caused by arsenic (As) poisoning, after consuming well water containing high levels of the mineral. Residents who had consumed well water containing 0.12 mg As/l for 10 years manifested arsenism with a prevalence rate of 1.4% of the city population. However, in residents who had consumed water containing 0.6 mg As/l for only 6 months, the prevalence rate increased to 47%, and the patients showed more severe symptoms.[1]

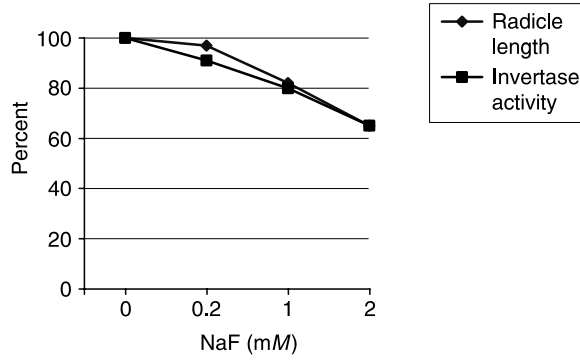

Figure 5.1 Effect of NaF on radicle growth and invertase activity in mung bean seedlings. *Source*: Ouchi, K., Yu, M.-H. and Shigematsu, A., *Fluoride*, 32, 171, 1999.

Plants exposed to different kinds of pollutants often show depressed growth or enzyme activity. For example, mung bean seedlings exposed to varying concentrations of sodium fluoride (NaF) for 3 days showed significant decreases in root elongation and the activity of invertase, a key enzyme responsible for the breakdown of sucrose into glucose and fructose. Invertase activity in seedlings exposed to 0.2, 1.0, and 2.0 m*M* NaF was decreased by 9, 22, and 41%, respectively, compared with the control treated with water. These results coincided with those for seedling growth (Figure 5.1).[2]

While it is true that exposure of organisms to sufficiently high levels of pollutants generally results in impaired growth or depressed enzyme activity, in a dose- or concentration-dependent manner, this is not always the case. Occasionally, under certain experimental conditions, increases in a certain endpoint (a measurable response of an organism to a stressor that is related to the evaluated characteristics chosen for assessing toxicity) may be observed in exposure studies where very low concentrations of toxicants are used. Increases in respiration (based on oxygen uptake by a tissue sample or an organism), activity of certain enzymes, and even growth rate, are some of the examples. Such observed increases have been interpreted as being due to an organism's effort to restore homeostasis by counteracting the stresses induced by toxicants. The effort nearly always requires additional energy expenditure and, therefore, increased metabolism of the exposed organism.

5.4 DURATION AND MODE OF EXPOSURE

The responses of an organism to stresses caused by toxicants are greatly affected by the duration of exposure. Ordinarily, one would expect long-term exposure to lead to a more severe injury than a short-term exposure. However, the dose or concentration of a toxicant is also important in determining the severity of the injury.

The mode of exposure of plants or animals to toxicants, continuous or intermittent, and the activity level of an exposed animal are also important in affecting pollutant toxicity. Normally, continuous exposure is more injurious than intermittent exposure, with other factors remaining the same. For instance, rats exposed to ozone (O_3) continuously for a sufficient period may develop pulmonary edema. However, when the animals are exposed to the same dose of O_3, but administered intermittently, no pulmonary edema may occur. A similar phenomenon can also occur with plants exposed to various kinds of air pollutants. One reason for this is that living organisms often can, to some extent, repair injuries caused by environmental chemicals. The magnitude of the health effects of O_3 on animals is also highly dependent on the activity level of the subject. Exercise increases the total volume of inhaled air, and so it will also increase the total dose of O_3 to the lung. The duration of the exercise is more important than the dose of the exposure.[3]

5.5 ENVIRONMENTAL FACTORS

Environmental factors, such as temperature, pH, humidity, and others, may affect pollutant toxicity in different ways. Some of these factors are examined in this section.

5.5.1 Temperature

Many reports have shown the effects of changes of temperature on living organisms.[4] Changes in ambient temperature affect the metabolism of xenobiotics in animals. For example, the rate at which chemical reactions occur increases with increase in temperature. With fish, an increase in temperature leads to faster assimilation of waste and therefore faster depletion of oxygen. Fish and other aquatic life can live only within certain temperature ranges. For metals, toxicity may increase with either an increase or decrease in ambient temperature.[5] Temperature also affects the response of vegetation to air pollution. Generally, plant sensitivity to oxidants increases with increasing temperature, up to 30°C. Soybeans are more sensitive to O_3 when grown at 28°C, regardless of exposure temperature or O_3 doses.[6]

5.5.2 pH

Maintenance of a particular pH in body fluids is critical for the well-being of animals and humans. The influence of pH on the toxicity of chemical agents varies according to the organism and the chemical agent. For instance, human body fluids must be maintained at very near to pH 7.4 for the body's metabolism to proceed properly, because most body enzymes function best when the pH remain around neutral. As noted in Chapter 4, the availability to plants of metals in the soil varies most markedly with soil pH. Increases in acidity (decreases in pHs) enhance the mobilization of metals in soil. Acid

precipitation, therefore, may greatly increase the availability of toxic metals, such as aluminum, to plants.

5.5.3 Humidity

The sensitivity of plants to air pollutants increases with increase in relative humidity. For instance, high relative humidity has been found to contribute to acute damage to forest vegetation caused by sulfur dioxide (SO_2).[7] Injurious effects of O_3 and nitrogen dioxide (NO_2) on vegetation have also been found to be greater when the relative humidity is high. A similar effect was found with fluoride toxicity, gladiolus plants exhibited a higher sensitivity to fluoride when relative humidity increased from 50 to 80%.[8]

5.6 INTERACTION

The actions of individual toxicants are affected by many factors, such as portals of entry, mode, metabolism, and others described previously. However, organisms are generally exposed to a complex mixture of different pollutants. Simultaneous exposure to more than one toxicant can have a dramatic impact on the outcome of exposure. Toxicants may interact to produce additive, potentiation, synergistic, or antagonistic effects. The factors affecting the outcome of exposure are complex and include, among others, the characteristics of the chemicals and the physiological condition of the organism.

5.6.1 Synergism, Additive and Potentiation

An *additive* interaction occurs where the combined effects of two compounds are simply additive. *Synergism*, however, describes a combined toxicity that is greater than the simple additive effect of two compounds. *Potentiation* describes synergism where one compound is generally assumed to have little or no intrinsic toxicity when administered alone (with synergism, both compounds have appreciable toxicity when administered separately).

Smoking and exposure to asbestos, for example, may have a synergistic effect, resulting in increased lung cancer. The presence of particulate matter such as sodium chloride (NaCl) and SO_2, or SO_2 and sulfuric acid mist simultaneously, would have potentiation or synergistic effects on animals. Many insecticides exhibit synergism or potentiation. A recent study with female rats showed that when the animals were exposed to fluoride and benzene hexachloride (BHC) simultaneously, a synergistic effect occurred, causing decreasing red blood cell counts and relative weight of the ovary.[9]

Exposure of plants to both O_3 and SO_2 simultaneously is more injurious than exposure to either of these gases alone. Laboratory studies showed that a single exposure to O_3 at 0.03 ppm and to SO_2 at 0.24 ppm for 2 hours or 4 hours did not injure tobacco leaves. However, when the leaves were exposed to a mixture of O_3 at 0.031 ppm and SO_2 at 0.24 ppm for 2 hours, a

moderate (38%) injury to the older leaves of Tobacco Wel W3 occurred (Table 5.1).[10] Similarly, an additive effect was observed on yield depression of bush beans in solution culture exposed to 2×10^{-4} *M* cadmium (Cd) and 2×10^{-5} *M* nickel (Ni), whereas synergistic effects on yield depressions were observed in solution culture for 5×10^{-5} *M* zinc (Zn), 3×10^{-5} *M* Cu, and 2×10^{-5} *M* Ni.[11]

5.6.2 Antagonism

Antagonism refers to a situation in which the toxicity of two or more chemicals present (or administered in combination or sequentially) is less than would be expected were the chemicals administered separately. Antagonism may be due to chemical or physical characteristics of the pollutants, or it may be due to the biological actions of the chemicals involved. For example, the highly toxic metal Cd is known to induce anemia and nephrogenic hypertension, as well as teratogenesis, in animals. Zn and selenium (Se) act to antagonize the action of Cd. This appears to be due to inhibition of renal retention of Cd by Zn and Se.

Antagonism includes cases where the lowered toxicity is caused by inhibition or induction of detoxifying enzymes. For example, parathion is known to inhibit mixed-function oxidase (MFO) activity, while DDT and dieldrin are inducers. The induction of MFO activity may also protect an animal from the effect of carcinogens by increasing the rate of detoxification. Antagonistic effects on xenobiotic metabolism *in vivo* are also known in humans. Cigarette smoking affects the activities of various liver enzymes, and studies on the term placenta of smoking mothers have shown it to cause marked stimulation of aryl hydrocarbon hydroxylase and related activities. Physical means of antagonism can also exist. For example, oil mists have been shown to decrease the toxic effects of O_3 and NO_2 or certain hydrocarbons in experiments on mice. This may be due to the oil dissolving the gas and holding it in solution, or to the oil containing neutralizing antioxidants.

Table 5.1 Synergistic Effect of Ozone and Sulfur Dioxide on Tobacco Bel W3 Plants

	Pollutant (ppm)		
Duration (hours)	**O_3**	**SO_2**	**Leaf damage (%)**
2	0.03	0	0
2	0	0.24	0
2	0.031	0.24	38

Source: Menser, H.A. and Heggestad, H.E., *Science*, 153, 424, 1966.

5.7 BIOLOGICAL FACTORS

5.7.1 Plants

Plants exhibit marked differences in their susceptibility to different pollutants. Genetic variation is probably the most important factor affecting plant response to environmental pollutants. Response varies between species of a given genus and between varieties within a given species. Such variation is a function of the influence genetic variability has on morphological, physiological, and biochemical characteristics of plants. For instance, gladiolus is known to be extremely sensitive to fluoride, and different gladiolus varieties show different responses to fluoride. The susceptibility of different species of plants to different pollutants varies markedly. For example, DDT applied to soil at a rate of 50 μg/g inhibited germination, seedling height, and fresh and dry weight in oil seed plants, but had no effect on rice, barley, and mung bean plants. In the oil seed plants, the DDT exposure caused a reduction in cell number and length and inhibited ion uptake, especially potassium ions (K^+) and calcium ions (Ca^{2+}).[12]

It has been shown that the sensitivity of two onion cultivars to O_3 is controlled by a single gene pair. After exposure to O_3 the stomata of an O_3-resistant cultivar were found to be closed, with no appreciable injury, whereas the stomata of O_3-sensitive cultivar remained open, with obvious injury.[13] The sensitivity of plants to air pollutants is also affected by leaf maturity. Generally, young tissues are more sensitive to peroxyacyl nitrate (PAN) and hydrogen sulfide (H_2S), and maturing leaves are most sensitive to the other airborne pollutants. According to Linzon,[7] in white pine the greatest chronic injury occurred in second-year needles exposed to SO_2.

5.7.2 Animals and Humans

Genetics, development, health status, gender, and behavior are among the most important factors that affect the response of animals and humans to pollutant toxicity.[5]

5.7.2.1 Genetic Factors

Not all organisms, including humans, react in the same way to a given dose of an environmental pollutant. In animal experiments, variation between species, as well as variation between strains within the same species, occurs. As shown in Table 5.2, the toxicity of the insecticides DDT and dieldrin differs markedly for different species. Substantial epidemiological data exist to illustrate that the interplay between environmental and genetic factors in the development of congenital malformations is significant. In animals, for example, it has often been shown that the rates of congenital anomalies differ between different strains of mice in response to the same dose of a teratogen.[14]

Table 5.2 Toxicity of DDT and Dieldrin

Compound	Organism	LD_{50} (mg/kg body weight)
DDT	Housefly	8
DDT	Bee	114
Dieldrin	Housefly	1.3
Dieldrin	Rat	87

In humans, such factors as serum, red blood cells, immunological disorders, and malabsorption can contribute to differences in individuals' response to stresses caused by environmental pollutants. For example, individuals with sickle-cell anemia are more susceptible to the effect of toxicants than are individuals without the anemia. People with malabsorptive disorders may also have problems; they may suffer nutritional deficiencies, which in turn may lead to an increased susceptibility to toxicants.

5.7.2.2 Developmental Factors

This category include aging, immature immune system, pregnancy, immature detoxification systems, and circadian rhythms. Examples of these factors, which all contribute to the varied responses to xenobiotics exhibited by individuals, include decline in renal function as a result of aging; lack of γ-globulin needed to cope with invading bacteria and viruses; lack of receptors needed in hormonal action; greater stresses encountered by pregnant women when metabolizing and detoxifying xenobiotics (not only for themselves but also for their fetuses), and immature hepatic MFO system in the young.

5.7.2.3 Diseases

Diseases in lungs, heart, kidneys, and liver predispose a person to more severe consequences of pollutant exposure. As mentioned previously, these organs are responsible for metabolism, storage, and excretion of environmental pollutants. Cardiovascular and respiratory diseases of other origins decrease an individual's ability to withstand superimposed stresses. An impaired renal function will certainly affect the kidneys' ability to excrete toxic substances or their metabolites. As noted earlier, the liver plays a vital role in detoxification of chemicals, in addition to its role in the metabolism of different nutrients and drugs. Disorders in the liver can therefore disrupt the proper detoxification process.

5.7.2.4 Behavioral Factors

Smoking, drinking, and drug habits are some examples of lifestyle choices that can affect an individual's response to toxicants. Smoking has been shown to act synergistically with several environmental pollutants. Asbestos workers or uranium miners who smoke are known to have higher lung cancer death rates

than those who do not smoke. Heavy drinking can lead to disorders in the brain and liver, a heavy drinker exposed to certain organic chemicals may therefore experience more serious liver injury than would a non-drinker.

5.7.2.5 Gender

The rate of metabolism of foreign compounds varies in animals and humans according to gender. The response to $CHCl_3$ exposure by laboratory mice, for example, shows a distinct sex variation. Male mice are highly sensitive to $CHCl_3$, and death often results following their exposure to this chemical.[15] The higher sensitivity exhibited by male mice to certain toxicants may be due to their inability to metabolize the chemicals as efficiently as female mice. It is interesting that the death rate of male mice exposed to $CHCl_3$ is also dependent on the strain of mouse. Studies have shown that the effect of BHC on the weights of rats' brains and kidneys varied with sex of the animal. The brain and kidney weights did not differ in male rats exposed to 25 ppm BHC from those of unexposed controls, but in female rats the weights of brain and kidney were both increased.

5.8 NUTRITIONAL FACTORS

5.8.1 Introduction

Results obtained from human epidemiological and animal experimental studies have clearly shown nutrition as an important factor affecting pollutant toxicity. For example, human populations exposed to environmental fluoride may or may not exhibit characteristic fluoride poisoning, depending on their nutritional status, such as the adequacy of protein, or vitamins A, C, D, or E. The interaction between nutrition and environmental pollutants is complex, and its study is a challenge to researchers in the fields of toxicology and nutrition – a new area of study called *nutritional toxicology* has emerged in recent years.

The relationship between nutrition and toxicology may include: the effect of nutritional status on the toxicity of environmental chemicals, the additional nutritional demands as a result of toxicant exposure, and the presence of toxic substances in foods.[16] Generally, nutritional modulation can alter rates of absorption of environmental chemicals, and therefore affect the circulating levels of those chemicals. Nutrition modulation can also induce changes in body composition, which in turn may result in altered tissue distribution of chemicals. Dietary factors can also influence renal function and pH of body fluids with altered toxicity. In addition, modified nutritional status of an individual may alter the responsiveness of the target organ.

5.8.2 Fasting and Starvation

Fasting or starvation, the most severe forms of nutritional modulation, influence xenobiotics toxicity in such a way that they may cause depressed metabolism and so reduced clearance of chemical agents. Consequently, increased toxicity may be seen.

Studies with animals have shown that the effect of fasting on microsomal oxidase activity is species-, substrate-, and sex-dependent. For instance, some reactions are decreased in male rats but increased in female rats, while others may not be affected at all. It is thought that the sex-dependent effect is related to the ability of androgen to enhance binding of some substrates to cytochrome P450. Animal studies also showed that glucuronide conjugation was decreased under starvation.

5.8.3 Proteins

The effects of proteins on the toxicity of environmental chemicals include both quantitative and qualitative aspects. Laboratory animals fed low-protein diets and exposed to toxicants often show higher toxic effects than observed in animals fed normal-protein diets. Protein deficiency causes hypoproteinemia and impaired hepatic function, leading to decreased levels of hepatic proteins, DNA, and microsomal P450, as well as lowered plasma binding of xenobiotics. Plasma contains many different proteins, such as albumin, glycoprotein, and lipoprotein. Albumin, in particular, has an important role in the binding and distribution of xenobiotics in the body, and so lowered binding of xenobiotics by plasma albumin could result in greater toxicity.

Protein deprivation may impair the metabolism of toxicants that occur in the body. Increased toxicity of chemical compounds and drugs in protein deficiency has long been known. The toxicity of most pesticides, such as chlorinated hydrocarbons, herbicides, fungicides and acetylcholinesterase (AChE) inhibitors, is increased by protein deficiency (Table 5.3). In a recent study, Tandon et al.[17] showed that the activities of the antioxidant enzymes, including superoxide dismutase (SOD), glutathione (GSH) peroxidase (GSHPx), and catalase, were decreased in rats fed a low-protein diet (containing 8% protein). Furthermore, the rats showed significantly increased levels of lipid peroxidation.

Alteration of xenobiotic metabolism by protein deprivation may lead to either enhanced or decreased toxicity, depending on whether the metabolites are more or less toxic than the parent compounds. The results shown in Table 5.3 reveal that low protein diets can cause decreased metabolism but increased mortality with respect to the chemicals concerned. In contrast, rats treated under the same conditions showed a decrease in mortality with respect to heptachlor, CCl_4, and aflatoxin B_1 (AFB_1), a toxin produced by *Aspergillus flavus*. It is known that heptachlor and AFB_1 are metabolized in the liver to their respective epoxide forms (Figure 5.2 and Figure 5.3), which are more toxic than the parent substances. For example, the epoxide form of AFB_1,

Table 5.3 Effect of Protein on Pesticide Toxicity[a]

	LD_{50} (mg/kg body weight)	
	Diet 3.5% casein	**Diet 26% caesin**
Chlorinated hydrocarbons		
DDT	45	481
Chlordane	137	217
Toxaphene	80	293
Endrin	6.69	16.6
Organophosphates		
Parathion	4.86	37.1
Malathion	759	1401
Herbicide and fungicides		
Diuron	437	2390
Captan	480	12,600

[a]Male rats fed for 28 days from weaning on diets of varying casein contents.
Source: Tandon, A., Dhawan, D.K. and Nagpaul, J. P., *J. Appl. Toxicol.*, 18, 187, 1998.

AFB_1-exo-epoxide, produces DNA adducts by binding to guanine.[18] As mentioned in the previous chapter (Reaction 4.5), CCl_4 is metabolized to $^{\cdot}CCl_3$, a highly reactive free radical.

In addition to the quantity, the quality of protein in diets also affects biotransformation. Experiments indicated a lowered microsomal oxidase activity in animals fed proteins of low biological value. When dietary proteins were supplemented with tryptophan, an essential amino acid, the enzyme activity was enhanced. Recent studies showed that mice exposed to NaF (5 mgF per kg body weight) exhibited significant decreases in DNA and RNA levels in the ovary and uterus. Administration of two amino acids, glycine and glutamine, alone and in combination, ameliorated the toxicity of NaF.[19]

Although protein nutrition has an important effect on pollutant toxicity, it should be pointed out that severely limited protein intake in humans is usually accompanied by inadequate intake of all other nutrients. Hence, it is often difficult to identify specific pathological conditions associated specifically with protein deficiency.

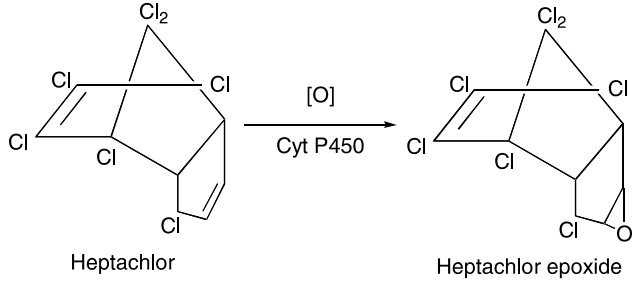

Figure 5.2 Formation of heptachlor epoxide.

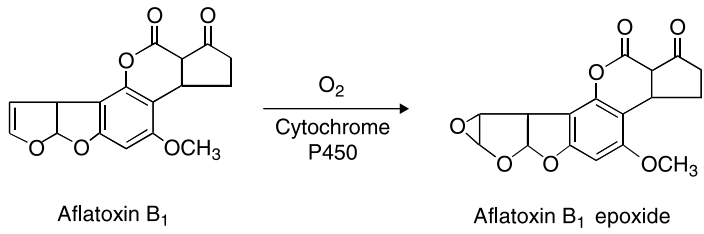

Figure 5.3 Formation of aflatoxin B1 epoxide.

5.8.4 Carbohydrates

A high-carbohydrate diet usually leads to a decreased rate of detoxification. Microsomal oxidation is generally depressed when the carbohydrate to protein ratio is increased. In addition, the nature of the carbohydrates also affects oxidase activity. For example, sucrose gives rise to the lowest activity, cornstarch, the highest value, and glucose and fructose give intermediate values. Since dietary carbohydrates influence body lipid composition, the relationship between carbohydrate nutrition and toxicity is often difficult to assess. However, environmental chemicals can affect, and be affected by, body glucose homeostasis in several different ways. For example, CCl_4 rapidly deactivates hepatic glucose 6-phosphatase by damaging the membrane environment of the enzyme. Trichloroethylene and several other compounds that are metabolized by the liver to glucuronyl conjugates are more hepatotoxic to fasted animals than to fed animals.

5.8.5 Lipids

Dietary lipids may affect the toxicity of environmental chemicals by delaying or enhancing their absorption. The absorption of lipophobic substances is delayed and that of lipophilic substances accelerated. The endoplasmic reticulum contains high levels of lipids, especially phospholipids (which are rich in polyunsaturated fatty acids). Lipids may influence the detoxification process by affecting the cytochrome P450 system because phosphatidylcholine is an essential component of the hepatic microsomal MFO system. A high-fat diet may cause more oxidation to occur because it may contribute to more incorporation of membrane material.

The type of lipid can also affect toxicant metabolism, as a high proportion of phospholipids are unsaturated due to the presence of linoleic acid (18:2) in the β-position of triacylglycerol. Dietary 18:2 is important in determining the normal levels of hepatic cytochrome P450 concentration and the rate of oxidative demethylation in rat liver.

Dietary lipids play a unique role in the toxicity of chlorinated hydrocarbon pesticides. Dietary lipids may favor more absorption of these pesticides, but once these chemicals are absorbed into the body they may be stored in the

adipose tissue without manifestation of toxicity. For this reason, obesity in humans is considered protective against chronic toxicity of these chemicals. Similarly, the body fat in a well-fed animal is known to store organochlorine pesticides. Fat mammals, fish, and birds are thus more resistant to DDT poisoning than their thinner counterparts. In times of food deprivation, however, organic chemicals, such as DDT and PCB, may be mobilized from their fat deposits and reach concentrations potentially toxic to the animal.

A recent report by the U.S. Institute of Medicine (IOM), stating the need to reduce saturated fat intake among the population as a means of reducing human exposure to dioxins, raises another concern about the toxicants. The report points out that saturated fats are a key source of human exposure to dioxins. Dioxins are a collection of more than 200 related compounds that may be linked to hormonal changes, neurodevelopmental problems in children, and cancer, in addition to other effects. They are ubiquitous agents that contaminate food as they cycle through the biosphere. Because dioxins are lipid soluble, they accumulate in many varieties of foods. According to the IOM, saturated fats in meat, dairy products, and certain species of fish are the biggest sources of human exposure to these chemicals.[20]

The role of dietary lipids in affecting pollutant toxicity has been fairly well defined for a few specific chemicals, including lead (Pb), fluoride, and hydrocarbon carcinogens. For example, high-fat diets are known to increase Pb absorption and retention. Moreover, competitive absorption of Pb and calcium (Ca) also occurs, which is probably due to competition for the Ca-binding protein (CaBP) whose synthesis is mediated by vitamin D, a fat-soluble vitamin. Studies have shown that a high-fat diet causes increased body burden of fluoride, resulting in higher toxicity. This is attributed to the delay of gastric emptying caused by high fat levels. Consequently, enhanced fluoride absorption may occur, leading to increased body burden of fluoride. Dietary fat does not increase metabolic toxicity of fluoride itself, however. As is well-known, AFB_1 is a potent liver cancer-causing agent. A high-fat diet offers protection from lethal effects of the toxin, presumably through dissolution of the carcinogen.

5.8.6 Vitamin A

Many reports describe vitamin A and its synthetic analogues as a potential factor in the prevention and treatment of some cancers. There is growing evidence that vitamin A may also alleviate pollutant toxicity. Epidemiological studies using a cohort of 8000 men showed a low incidence of lung cancer in those with a high level of vitamin A in their diet, while incidence was higher in individuals with a diet with low levels of vitamin A . In experimental studies, rats exposed to PCB, DDT, and dieldrin showed a 50% reduction in the liver vitamin A store. In other studies, rats deficient in vitamin A exhibited lowered cytochrome P450 activity in the liver. The effect of vitamin A deficiency on MFO enzymes, however, depends on several factors, such as substrate, tissue, and animal species. Recent studies have demonstrated that rats exposed to

fluoride show increased levels of lipid peroxide (LPO) in the liver, serum, heart, and kidneys, whereas the activities of SOD and GSHPx and the levels of GSH were decreased. Administration of β-carotene (which can be partially converted to vitamin A in the body) reduced LPO levels while raising SOD activity.[21]

The mechanism involved in vitamin A action relative to carcinogenesis may in part involve a free-radical scavenging action of the vitamin. Because vitamin A is required in the differentiation of epithelial cells (important in both respiratory and gastrointestinal tracts), its deficiency may affect transformation of epithelia and thus predispose the tissue to neoplastic changes.

5.8.7 Vitamin D

The role that vitamin D plays in the prevention of rickets and osteomalacia has been well documented. To play its role in the maintenance of Ca homeostasis, vitamin D must be converted into its metabolically active form, 1,25-dihydroxy-D_3 (the hormone-like substance). Vitamin D_3 (cholecalciferol) is first hydroxylated in the liver to 25-hydroxy-D_3. The resultant 25-hydroxy-D_3 is then converted in the kidney to 1,25-dihydroxy-D_3, the active form of the vitamin. The 25-hydroxylation of cholecalciferol requires NADPH, O_2, and an enzyme whose properties are similar to those of microsomal MFO.[22] In addition, 25-hydroxy-D_3 has been shown to competitively inhibit some cytochrome P450 reactions *in vitro*. Patients suffering from drug-induced osteomalacia show increased rates of catabolism of vitamin D_3 to 25-hydroxy-D_3. In a recent laboratory study of male mice exposed to NaF, vitamin D, alone or in combination with vitamin E, was found to ameliorate the adverse effect of NaF on reproductive function and fertility.[23]

5.8.8 Vitamin E (α-tocopherol)

Vitamin E, a membrane-bound antioxidant and free-radical scavenger, appears to offer protection against injuries caused by O_2, O_3, and NO_2, and nitrosamine formation. Male rats administered daily doses of 100 mg tocopheryl acetate and exposed to 1.0 ppm O_3 were shown to survive longer than rats deficient in vitamin E. The action of O_3 is attributed in part to formation of free radicals. Vitamin E is also believed to protect phospholipids of microsomal and mitochondrial membranes from peroxidative damage by reacting with free radicals (Figure 5.4). Because lipid peroxidation is associated with decrease in oxidase activities, it is expected that the enzyme activity is affected by dietary vitamin E. Maximum activity has been observed when diets include both polyunsaturated fatty acids and vitamin E.

Nitrosamine, known to be carcinogenic, leads to liver cancer. The interaction between vitamin E and nitrosamines is attributed to the inhibitory effect of the vitamin on nitrosamine formation, i.e., vitamin E competes for nitrite, a reactant in nitrosamine formation.

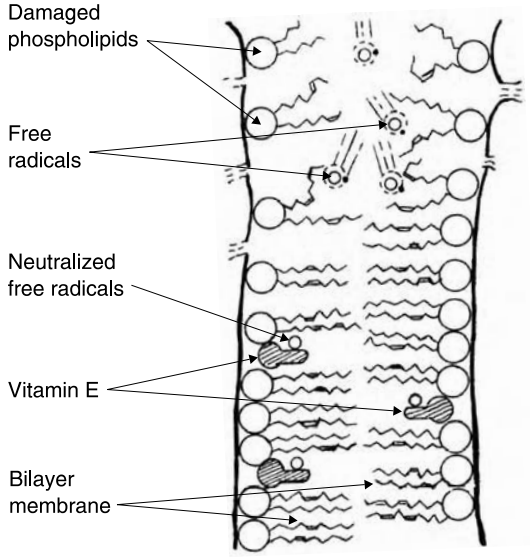

Figure 5.4 Action of vitamin E to stop free-radical-induced chain reactions in microsomal and mitochondrial membrane.

Laboratory studies with isolated rat hepatocytes showed that cellular α-tocopherol maintains the viability of the cell during a toxic insult.[24] A recent study showed that male mice treated with NaF (10 mgF per kg body weight) exhibited changes in epididymal milieu, as revealed by significant decreases in levels of sialic acid and protein and ATPase activity in epididymides. These changes in turn disrupted the sperm maturation process, leading to a significant decline in cauda epididymal sperm count, motility and viability. Consequently, a significant decline in fertility rate occurred. Withdrawal of NaF treatment for 30 days produced incomplete recovery. However, vitamin E supplementation during the withdrawal period resulted in recovery of all NaF-induced adverse effects.[25]

5.8.9 Vitamin C (Ascorbic acid)

Vitamin C is found in varying amounts in almost all animal and human body tissues. In humans, high vitamin C levels occur particularly in adrenal and pituitary glands, eye lens, and various soft tissues. Vitamin C is a potent antioxidant and participates in many cellular oxidation–reduction reactions. Vitamin C-deficient guinea pigs have been shown to exhibit an overall deficiency in drug oxidation, with marked decreases in N- and O-demethylations, and in the contents of cytochrome P450 and cytochrome P450 reductase.[16] Administration of ascorbate to the deficient animals for 6 days reversed these losses of MFO activity.

The effect of vitamin C appears to be tissue-dependent.[25] Epidemiological studies show that persons with high intakes of dietary vitamin C or citrus fruit have a lower than normal risk of developing cancer. Cancer prevention by vitamin C is thought to be mainly due to its role as an antioxidant and free-radical scavenger. Oxidative and free-radical-induced damage to DNA and cell membranes has been considered as the most important factor in cancer initiation – substantial evidence indicates that vitamin C can help prevent such damage.[26]

A variety of experimental tumors of the gastrointestinal tract, liver, lung, and bladder can be produced by nitroso compounds.[27,28] Nitroso compounds are produced by the reaction of nitrite with secondary and tertiary amines, amides or others, as shown below (Reaction 5.1):

$$\begin{array}{l} \mathrm{R} \\ | \\ \mathrm{NH} \\ | \\ \mathrm{R} \end{array} + \mathrm{HNO_2} \rightarrow \begin{array}{l} \mathrm{R} \\ | \\ \mathrm{N-N=O} \\ | \\ \mathrm{R} \end{array} + \mathrm{H_2O} \qquad (5.1)$$

The nitrosation of several secondary and tertiary amines can be blocked *in vitro* by the addition of vitamin C. The vitamin appears to compete for the nitrite, thus inhibiting nitrosation. It has been demonstrated that vitamin C does not react with amines, nor does it enhance the rate of nitrosamine decomposition. However, it reacts very rapidly with nitrite and nitrous acid. The vitamin appears to decrease the available nitrite by reducing nitrous acid to nitric oxide (Reaction 5.2), leading to inhibition of the nitrosation reaction.

$$\text{Ascorbate} + 2\mathrm{HNO_2} \rightarrow \text{Dehydroascorbate} + 2\mathrm{NO} + 2\mathrm{H_2O} \qquad (5.2)$$

Vitamin C has been shown to prevent growth retardation and severe anemia in young Japanese quail exposed to Cd.[29] Vitamin C, with vitamin E, has been shown also to protect against herbicide-induced lipid peroxidation in higher plants. Cell damage is markedly increased in plants that have a much lower or a much higher than normal ratio of vitamin C to vitamin E concentrations (10 to 15:1, w/w) or a lower amount of both vitamins.[30]

The average American is thought to ingest approximately 70 μg Cd, 0.9 μg As and 4.1 mg nitrite per day, as well as being exposed to ambient air containing carbon monoxide (CO), O_3, Pb, cigarette smoke, and other materials.[31] In view of the many vital functions that vitamin C performs in biological systems, and of the increasing exposure of people to various drugs and xenobiotics, some researchers have suggested that the recommended dietary allowances (RDA) for ascorbic acid may be inadequate.[32] In support of the suggestion is the result of a recent study on urban air pollution. The study showed that short-term exposure produced some decrease in lung function, which might be counteracted by pretreatment with vitamin C.[33] In a separate study on mice, fluoride has been shown to impair the protective enzymes, such as SOD, GSHPx, and catalase, thereby increasing ovarian LPO and injury.

Vitamins C and E were shown to be beneficial in the amelioration of the detrimental effects induced by fluoride.[34]

The most outstanding chemical characteristics of the ascorbate system (ascorbic acid/ascorbate, ascorbate free radical, dehydroascorbic acid) are its redox properties. Ascorbate is a reactive reductant, but its free radical ($A^{\cdot -}$) is relatively nonreactive. Interestingly, there is evidence that vitamins E and C probably act synergistically, i.e., vitamin E acts as the primary antioxidant (particularly in biomembranes) and the resulting vitamin E radical ($E^{\cdot}$) then reacts with ascorbate (AH^-) to regenerate vitamin E,[35] as shown in Reaction 5.3.

$$\text{vitamin E}^{\cdot} + AH^- \rightarrow \text{vitamin E} + A^{\cdot -} \tag{5.3}$$

The interaction between vitamin $E^{\cdot}$ radicals and ascorbate in protecting against potentially damaging organic free radicals is illustrated in Figure 5.5.

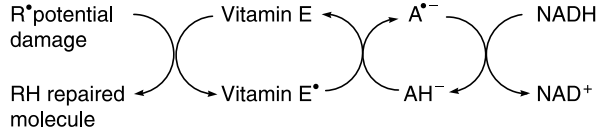

Figure 5.5 Interaction of vitamin C (ascorbate) with vitamin E.

5.8.10 Minerals

Mineral nutrition influences toxicology in different ways. Interactions are the rule rather than the exception when considering the effects of trace nutrients on detoxification. As with the macronutrients, trace mineral elements can influence absorption of xenobiotics. Divalent cations can compete for chelation sites in intestinal contents, as well as for binding sites on transport proteins. It is widely known that competitive absorption of Pb and Ca occurs, which is probably due to competition for binding sites on intestinal mucosal proteins mediated by vitamin D. However, Zn is known to provide protection against Cd and Pb toxicities.[36] Absorption of Zn is facilitated by complexing with picolinic acid, a metabolite of the amino acid tryptophan. Although both Cd and Pb form complexes with picolinic acid, the resulting complexes are less stable than the Zn complex. Se is antagonistic to both Cd and mercury (Hg), thus reducing their toxicity. In addition, Se enhances vitamin E function in the prevention of lipid peroxidation. The mechanisms involved in the functioning of Se and vitamin E are, however, different. Whereas α-tocopherol functions as a membrane-bound antioxidant, acting as a free-radical scavenger, Se participates at the active site of GSHPx, and is thus part of the enzyme. GSHPx protects membrane lipids by catalyzing the destruction of H_2O_2 and organic hydroperoxides before they cause membrane disruption.

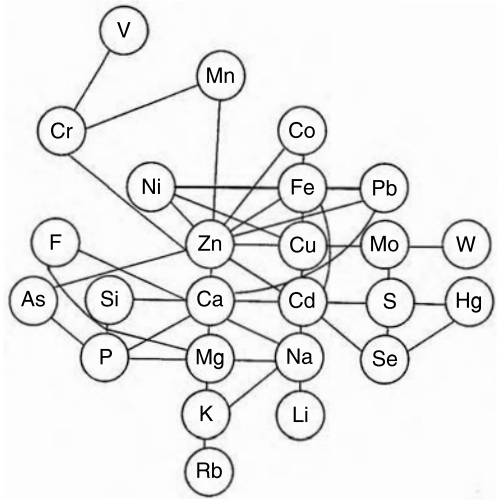

Figure 5.6 Interaction between mineral elements.

Since cytochrome P450 requires iron (Fe) for its biosynthesis, deficiency of Fe may lead to depressed MFO activity. Dietary Fe deficiency in rats has been shown to result in a rapid loss of the cytochrome P450 content and MFO activity in the villous cells of duodenal mucosa.[37] As noted earlier, rats fed a low-protein diet exhibited increased levels (56%) of LPO and decreased activities of antioxidant enzymes, such as SOD, GSHPx, and catalase. When lithium (Li) (as carbonate) was administered to rats fed a low-protein diet, the activity of GSHPx was increased, while the activities of catalase and SOD were brought to within normal limits. Furthermore, Li treatment diminished the increase in LPO level.[17]

Dietary magnesium (Mg) and potassium (K) restriction have recently been shown to enhance the toxicity of paraquat (an organic herbicide) in rats.[38] The main mechanism involved in paraquat toxicity is tissue oxidation by reactive oxygen radicals generated by redox cycling of the compound.[39] Rats fed a Mg-restricted diet and exposed to paraquat exhibited a severe toxicosis, whereas those with a K-restricted diet showed a mild toxicosis. Restriction of Mg and K was shown to have a synergistic effect on paraquat-dependent toxicosis.[37] Figure 5.6 shows the interaction among mineral elements.

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5.10 REVIEW QUESTIONS

1. Explain how protein nutrition may affect the body's response to environmental chemicals.
2. What is meant by oxygen stress?
3. What are antioxidants? Give four examples of both endogenous antioxidants and antioxidant enzymes system.

4. Explain how vitamins C and E act as free-radical scavengers. What are the main differences between vitamins C and E when they act as free-radical scavengers?
5. Which is generally more injurious to an organism exposed to a toxicant, a continuous exposure or an intermittent exposure?
6. Explain the differences between synergism and antagonism. Also, explain how Zn and Cd may interact.
7. What are the reasons for old and young people being more susceptible than adults to toxicant-induced injury?
8. How may nutrition generally affect toxicology?
9. Explain how a person's protein nutrition may affect his or her response to xenobiotics.
10. What role do dietary lipids play in affecting the toxicity of organochlorine pesticides?
11. Explain the relationship between vitamin A and fluoride-induced toxicity.
12. Explain the role that vitamin E plays in lipid peroxidation.
13. What role do vitamins C and E play in nitrosation?
14. Why is Fe deficiency related to the MFO system?
15. Explain the relationship between a low-protein diet and the levels of antioxidant enzymes.
16. How does ascorbate interact with vitamin E free radicals?
17. What role does Se play in detoxification? Also, briefly explain the mechanism involved.

Chapter 6

Biotransformation – Metabolism of Xenobiotics

6.1 INTRODUCTION

Metabolism is defined as the sum of all chemical reactions that occur within a living cell. The purpose of cellular metabolism is to maintain the homeostasis of the cell within a population of other cells. *Homeostasis* refers to a tendency toward maintenance of a relatively stable internal environment in the bodies of higher animals through a series of interacting physiological processes. Metabolism is usually subdivided into two categories: anabolism and catabolism. *Anabolism* is the synthesis of larger molecules from smaller ones. The synthesis of a protein from its amino acid building blocks is an example. Anabolism generally requires input of energy from an energy source, such as ATP. *Catabolism* refers to the degradation of larger molecules to smaller ones, e.g., the breakdown of starch to glucose. In higher organisms, catabolism of carbohydrates and fats results in the production of ATP.

Following their absorption into a mammal, xenobiotics are subjected to metabolic conversion in the body, resulting in structural changes. This metabolic process is called biotransformation. Biotransformation may occur in any of several body tissues and organs, including skin, lung, intestine, liver, and kidney. The liver carries out the majority of the chemical reactions because it contains a large number of nonspecific enzymes capable of biotransformation of xenobiotics. The enzymes involved in the biotransformation are named *mixed-function oxidase* (MFO), commonly known as cytochrome P450. The liver metabolizes not only many xenobiotics, but also drugs to which the body is exposed. Biotransformation in the liver is thus a critical process in the body's defense against the toxic effects of a wide variety of xenobiotics.[1]

6.2 TYPES OF BIOTRANSFORMATION

As mentioned in Chapter 4, the process of xenobiotic biotransformation consists of two phases: Phase I and Phase II (Figure 6.1). *Phase I* biotransformation includes oxidation, reduction, and hydrolysis. *Phase II* biotransformation is essentially composed of conjugation reactions.

Among the representative oxidation reactions catalyzed by cytochrome P450 are hydroxylation (of an aliphatic or aromatic carbon), dealkylation (including atoms such as O, S, or N), deamination, epoxidation, oxidative

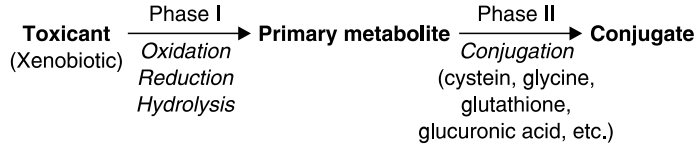


Figure 6.1 The two phases of biotransformation.

group transfer, and dehydrogenation. Occasionally, hydroxylation is treated as an independent reaction system. Dealkylation produces an aldehyde, whereas deamination produces an ammonia or an amine, and the primary metabolite (Figure 6.2).

Xenobiotics containing an aldehyde, ketone, disulfide, sulfoxide, azo, nitro group, and others, as well as certain metals or metalloids, are often reduced *in vivo*. Several endogenous reducing agents are involved in the reduction processes, including the reduced forms of glutathione (GSH), flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (phosphate) (NAD(P)). Figure 6.2 shows examples of azo-reduction and nitro-reduction.

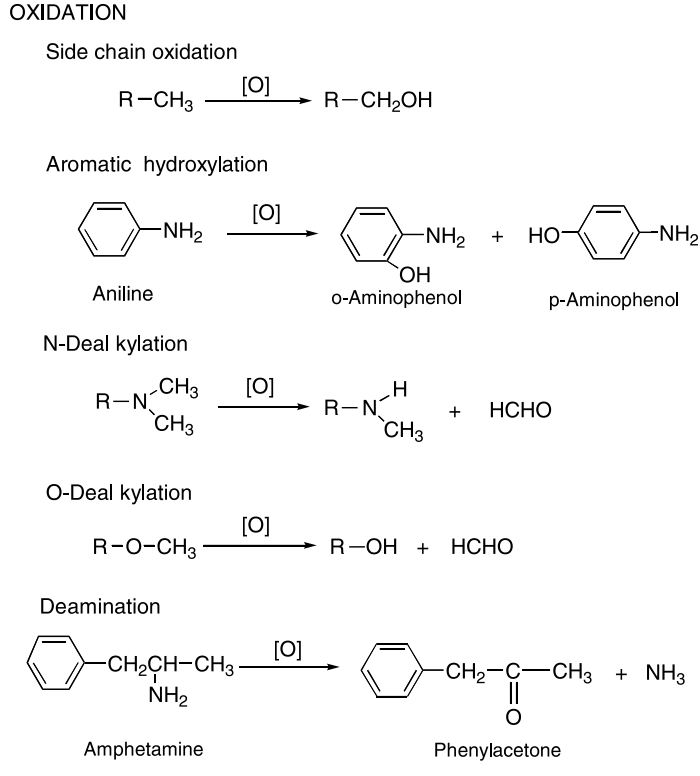


Figure 6.2 Examples of Phase I biotransformation reactions.

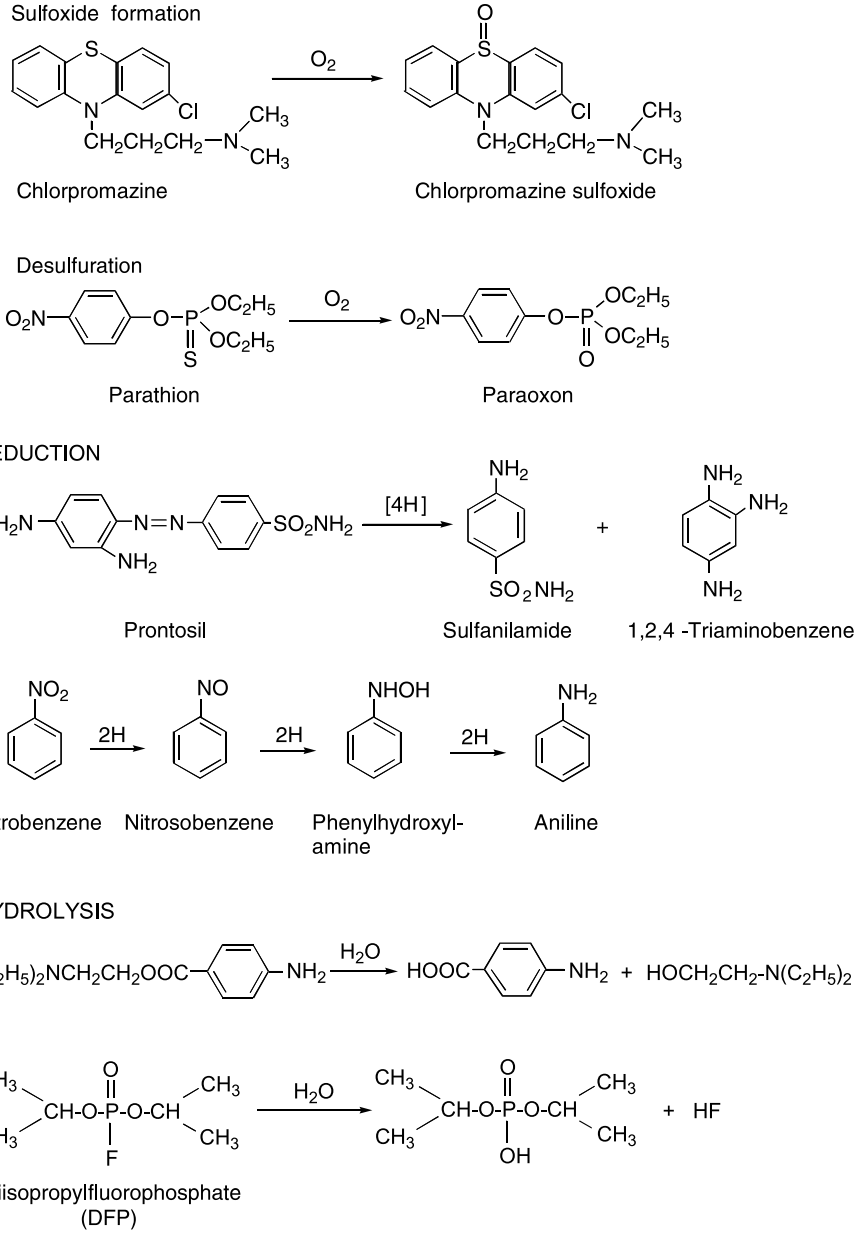


Figure 6.2 Continued.

In hydrolysis, splitting of ester and amide bonds is common. Hydrolytic enzymes contained in mammals include carboxylesterases, cholinesterases, and organophosphatases. These are involved in hydrolyzing functional groups, such as carboxylic acid ester, phosphoric acid ester, and acid anhydride (e.g., diisopropylfluorophosphate (DFP)) (Figure 6.2).

6.3 MECHANISM OF BIOTRANSFORMATION

In the two phases of biotransformation shown in Figure 6.2 and Figure 6.3, a lipophilic foreign chemical is first oxidized in a Phase I reaction so that a functional group, such as –OH, $–NH_2$, –COOH, or –SH, is introduced into the molecule, forming a product called a *primary metabolite*. A slight increase in hydrophilicity usually occurs as a result of the reaction.

Phase II reactions, conversely, are synthetic or conjugation processes. Here, a primary metabolite from Phase I biotransformation, or a parent xenobiotic, reacts with an endogenous substance and forms a conjugate (Figure 6.3). Included in this process are sulfation, acetylation, methylation, glucuronidation, and conjugation with GSH or amino acids. Most Phase II biotransforma-

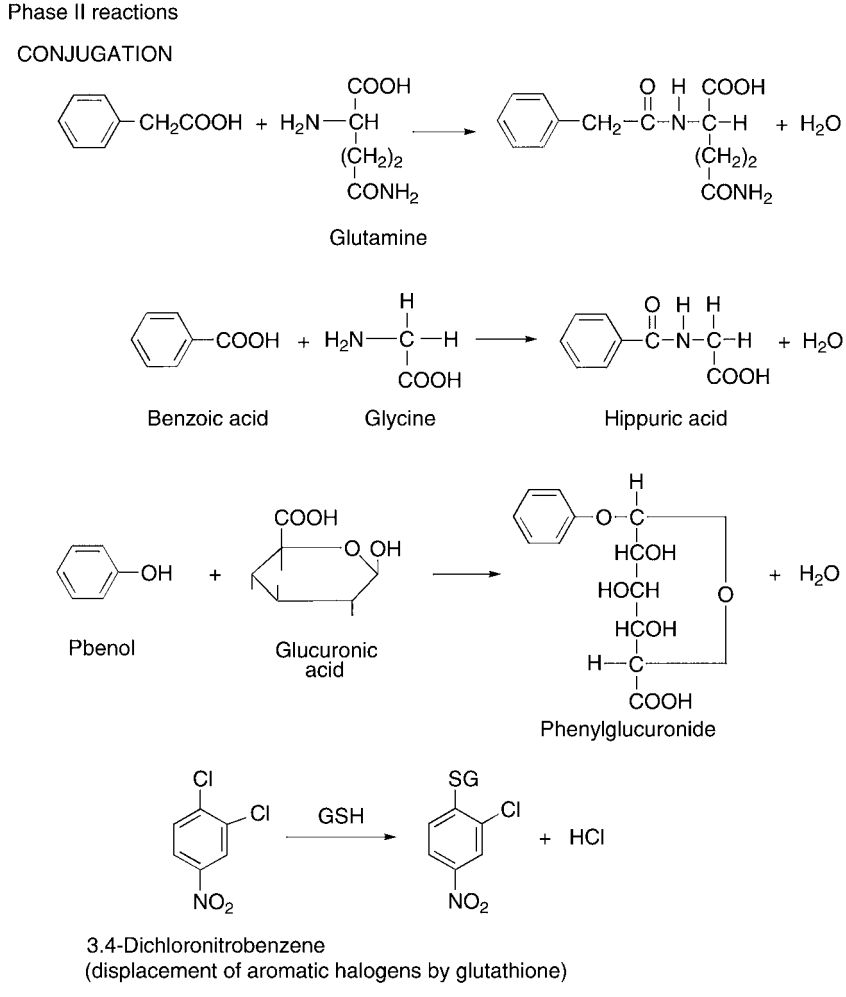


Figure 6.3 Examples of Phase II biotransformation reactions.

tion results in substantial increase in xenobiotic hydrophilicity, thus promoting the excretion of xenobiotics. Many xenobiotics are lipophilic and undergo Phase I and Phase II reactions sequentially, whereas others may participate in only one phase. In this case, a toxicant may combine directly with an endogenous substance, forming a conjugate. Endogenous substances known to participate only in Phase II reactions include glycine, cysteine, GSH, glucuronic acid, sulfates, and some other water-soluble substances. Several representative Phase II reactions are shown in Figure 6.3.

6.4 CHARACTERISTICS OF BIOTRANSFORMATION

The NADPH-cytochrome P450 system, commonly known as the *mixed-function oxygenase system* (*MFO system*), is the most important enzyme system involved in Phase I biotransformation. The cytochrome P450 system, localized in the smooth endoplasmic reticulum of cells of most mammalian tissues, is particularly abundant in the liver. Contrary to most enzymes, which catalyze the metabolism of one substrate with one mechanism, quickly and efficiently, the cytochrome P450 system contains a number of isozymes – multiforms of an enzyme that are structurally equivalent but catalytically distinct from one another – that can catalyze a variety of substrates with multiple mechanisms, slowly and inefficiently (average turnover rate is one per minute). The reactions that the isozymes catalyze include aliphatic or aromatic hydroxylation, epoxidation of a double bond, N-oxidation, sulfoxidation, dealkylation, deamination, dehydrogenation, dehalogenations, oxidative group transfer, and cleavage of esters (Figure 6.2).[2]

During the catalytic reaction, the oxidized form of an iron atom (Fe^{3+}) at the active site of cytochrome P450 binds directly to the substrate (XH) (Figure 6.4). Reduction of this enzyme–substrate complex follows, with an electron being transferred from NADPH via NADPH cytochrome P450 reductase. The reduced (Fe^{2+}) enzyme–substrate complex binds molecular oxygen (O_2), and is reduced further by a second electron (presumably donated by NADH via cytochrome b_5 and NADH cytochrome b_5 reductase). The enzyme–substrate–oxygen complex splits into oxidized substrate, water, and the oxidized form of the enzyme. The overall reaction by which a substrate or an environmental chemical, XH, is oxidized by the cytochrome P450 system is shown in Reaction 6.1:

$$\mathrm{XH\ (substrate)} + \mathrm{O_2} + \mathrm{NADPH} + \mathrm{H^+} \rightarrow \mathrm{XOH\ (product)} + \mathrm{H_2O} + \mathrm{NADP^+} \tag{6.1}$$

As shown in Reaction 6.1, one atom from O_2 is reduced to water and the other is incorporated into the substrate, producing ROH, a hydroxylated metabolite. The constituents required in this enzyme system are O_2, NADPH, and magnesium ions (Mg^{2+}).

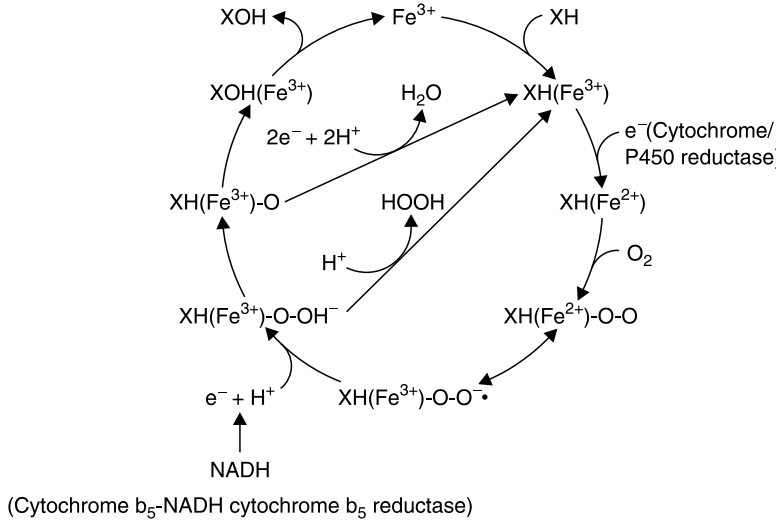


Figure 6.4 The cytochrome P450 catalytic cycle.

Carbon monoxide (CO) readily binds the reduced form of the cytochrome, forming a complex with a maximum absorption at 450 nm. (This is the origin of the name of the enzyme cytochrome P450.) Formation of the CO-complex results in inhibition of enzyme activity and thus the oxidation process.

Unlike the cytochrome P450 system, most hepatic Phase II enzymes are located in the cytoplasmic matrix. For the biotransformation reactions to proceed properly, each of the participating enzymes must function efficiently. It is also obvious that sufficient intracellular content of cofactors is required for one or more reactions. Required cofactors include NADH, NADPH, O_2, glucose 1-phosphate, glucuronate, ATP, cysteine, and GSH.

6.5 CONSEQUENCE OF BIOTRANSFORMATION

Removal of xenobiotics from a biological system is carried out primarily by biotransformation and excretion mechanisms. Some xenobiotics, especially the lipophilic ones, are readily reabsorbed by the kidney cells. Unless the chemicals are converted to more polar metabolites, they will remain in the body, mostly in the fatty tissues, for a long period. As described in Section 6.3, the resultant products from biotransformation are usually, but not always, more hydrophilic or polar than the parent compound, and thus more readily excreted.

6.5.1 Biotransformation of Endogenous Substances

Although hepatic enzymes that catalyze biotransformations are responsible for the conversion of xenobiotics, they also participate in the catabolism, or

breakdown, of endogenous substances. For example, the hormone testosterone is deactivated by cytochrome P450. The S-methylases detoxify hydrogen sulfide (H_2S) formed by anaerobic bacteria in the intestinal tract. (It follows that chemicals or conditions that influence the activity of Phase I and Phase II enzymes can affect the normal metabolism of endogenous substances.)

6.5.2 Activation of Xenobiotics

Although the biotransformation of lipophilic xenobiotics often results in the production of a more stable, water-soluble, and more readily excretable metabolite, the activation of xenobiotics also occurs. In other words, certain xenobiotics may be converted through biotransofrmation to reactive electrophilic species that are more potent than the parent compounds. For example, the biotransformation of benzo[a]pyrene (BaP), which is both mutagenic and carcinogenic, involves several steps, including the formation of BaP-7,8-epoxide and BaP-7,8-diol, before being converted to the final product BaP-7,8-dihydrodiol-9,10-epoxide (Figure 6.5a). The resultant BaP-7,8-dihydrodiol-9,10-epoxide is more active than BaP itself, because it can readily combine with guanine to form an adduct. Similarly, aflatoxin B_1 is toxic because the metabolite aflatoxin B_1 epoxide can cause liver cancer (see Section 5.8.3). Another example is carbon tetrachloride (CCl_4). This compound is hepatotoxic because, following biotransformation, it is converted to a trichloromethyl free radical (Reaction 4.5), which binds to protein and initiates lipid peroxidation (Figure 6.5b).

The reactive chemical species produced during biotransformation must be metabolized; otherwise they may interact with a nucleophilic site in a vital cell

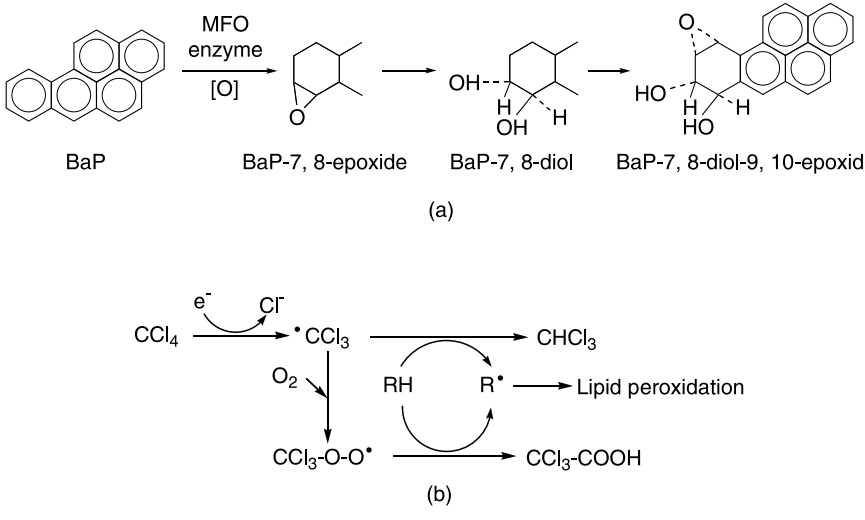


Figure 6.5 Activation of xenobiotics through biotransformation: (a) benzo[a]pyrene, and (b) CCl4.

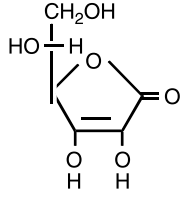
constituent and induce cellular damage.[3] As mentioned earlier, many of the reactive metabolites can bind covalently to macromolecules in liver cells. For instance, the hepatotoxic CCl_4 noted above can covalently bind to lipid components of the liver endoplasmic reticulum.[4] Some of the reactive electrophiles are also carcinogenic.

Although liver cells depend on detoxification enzymes for protection against the reactive electrophilic species produced during biotransformation, endogenous antioxidants, such as vitamin E (α-tocopherol) and tripeptide GSH (L-γ-glutamyl-cysteinyl-glycine) (Figure 6.6), also provide protection. Vitamin E is widely known as a free-radical scavenger. Its main role is to protect lipid material in membranes against free-radical-initiated peroxidation reactions (see sections 4.4 and 5.5.8). Experimental evidence indicates that livers of animals fed diets deficient in vitamin E were more vulnerable to lipid peroxidation following exposure to CCl_4 than those fed diets containing supplemental vitamin E.[4] GSH, conversely, has a nucleophilic sulfhydryl (–SH) group (Figure 6.6) that can react with, and thus detoxify, reactive electrophilic species.[5] GSH can also donate its sulfhydryl hydrogen to a reactive free radical. The resultant GSH radical ($GS^{\cdot}$) can react with another $GS^{\cdot}$, producing a molecule of stable glutathione disulfide (GSSG) (Reaction 6.2 and Reaction 6.3).

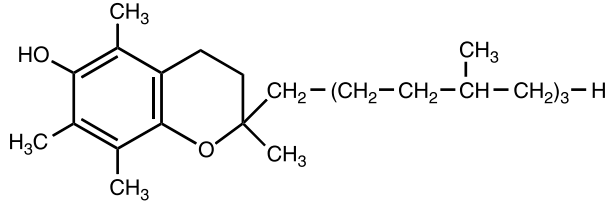
$$GSH + X^{\cdot} \rightarrow HX + GS^{\cdot} \tag{6.2}$$

$$GS^{\cdot} + GS^{\cdot} \rightarrow GSSG \tag{6.3}$$

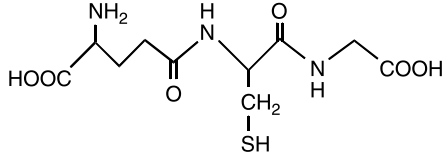
The resultant GSSG can be reduced back to GSH through a NADPH-dependent reaction catalyzed by glutathione reductase (Reaction 6.4). The NADPH is derived from reactions involved in the pentose phosphate pathway.



Ascorbic acid
(vitamin C)

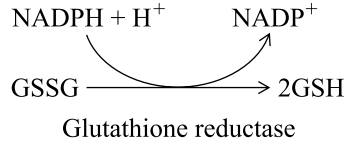


Vitamin E



Glutathione (GSH)

Figure 6.6 Examples of antioxidant chemical species.



(6.4)

In addition to antioxidant chemical species, such as vitamins E and C and GSH, there are several enzymes, called *antioxidant enzymes*, that play a pivotal role in the defense against free-radical-mediated cellular damage. These include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), and glutathione reductase. Figure 6.7 shows the interrelationship between these enzymatic components.

6.6 FACTORS AFFECTING BIOTRANSFORMATION

The activity and levels of each of the P450 enzymes have been shown to vary between individuals, depending on environmental and genetic factors. Increased P450 enzyme activity can result from: exposure to xenobiotics or other environmental factors that induce the synthesis of the enzyme, stimulation of preexisting enzyme by a xenobiotic, or gene duplication leading to over expression of a P450 enzyme. Decreased P450 enzyme activity can occur as a result of: exposure to a xenobiotic capable of inhibiting or inactivating a preexisting P450 enzyme, exposure to an environmental factor (such as a xenobiotic) that suppresses P450 enzyme expression, or a mutation that leads either to blocking the synthesis of a P450 enzyme or to synthesis of an enzyme with limited activity or total inactivity.[6]

For example, in animals many drugs and environmental chemicals can either stimulate or inhibit microsomal enzymes and so stimulate or inhibit biotransformation. Chemical agents that can stimulate enzymes include halogenated hydrocarbon insecticides, urea herbicides, polycyclic aromatic hydrocarbons (PAHs), nicotine and other alkaloids, and some food preservatives. Agents that can inhibit the enzymes include not only CO, as mentioned

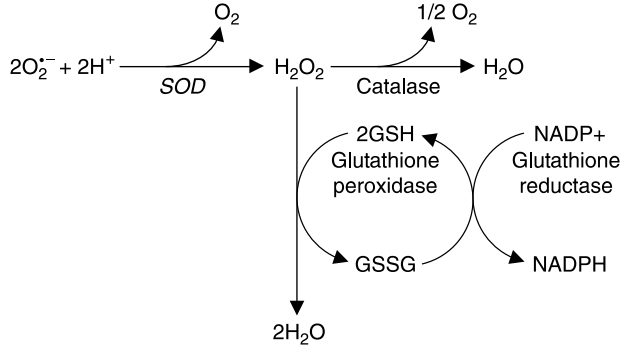


Figure 6.7 Examples of reactions involving antioxidant enzymes.

previously, but also ozone (O_3), CCl_4, and organophosphorus insecticides. The consequence of the actions of these chemicals on microsomal enzymes is complex and may include normal body constituents, such as steroid hormones, thyroxin, and bilirubin, or altered metabolism and action of drugs and carcinogens.

The health status of an individual, including nutritional status, also plays an important role in biotransformation. As noted earlier, several endogenous substances, such as glycine, glutamine, glucuronic acid, and GSH are necessary for conjugation with xenobiotics or their metabolites in Phase II biotransformation (Figure 6.3). Glycine and glutamine are both amino acids, GSH is a tripeptide derived from three amino acids, and glucuronic acid is formed from glucose that is derived directly from dietary source or derived from liver glycogen through glycogenolysis (breakdown of glycogen). It is therefore likely that the availability of proteins or liver reserves of glycogen may be insufficient in individuals with poor nutritional status or liver problems. These conditions can lead to impaired biotransformation.

The importance of ethoxyresorufin O-deethylase (EROD), one of the hepatic cytochrome P450-dependent mono-oxygenases, has become widely known, and is increasingly accepted as an indicator of exposure to common organic pollutants. Various organic chemicals, including chlorinated hydrocarbons, have been shown to adversely affect the enzymes involved in biotransformation. This is manifested in a study summarized in Case Study 6.1. The results in Case Study 6.1 suggest that pollutants such as PCBs may activate detoxification capacity, but weaken antioxidant status, in fish in a polluted river system.[7]

Case Study 6.1

A group of researchers collected brown bullheads (*Ameiurus nebulosus*) from the St. Lawrence River, a relatively polluted system, and compared various parameters in the fish with those in fish from Lac La Peche, a relatively non-polluted system in Canada (used as a reference).[7] The main results obtained were as below:

- The activities of liver ethoxyresorufin O-deethylase (EROD) – a common Phase I enzyme – in fish collected from the St. Lawrence River were significantly higher (2.8-fold) than those in fish from the reference site.
- The conjugation activity by hepatic glutathione S-transferase (GST) was three times higher in fish from the St. Lawrence River than in fish from the reference site.
- The content of PCBs in white muscle was 22 times higher in fish from the St. Lawrence River than in fish from the reference site.
- The activities of cytosolic SOD were significantly higher, while those of catalase in kidney and GSHPx in red and white muscles were lower, in the St. Lawrence River fish than those in the reference fish.
- The concentrations of total GSH in different tissues were significantly lower in liver, kidney, and white muscle of fish from the St. Lawrence River compared with those in fish from the reference site.

The important role that SOD plays in biotransformation is well recognized. Several factors can either enhance or inhibit the enzyme, fluoride being one of them. For example, both *in vivo* and *in vitro* studies show that the activity of SOD from the earthworm (*Eisenia fetida*) is inhibited by NaF in a concentration-dependent manner.[8] Other studies show that in laboratory animals exposed to NaF, both SOD and GSHPx activity decrease significantly.[9] However, in aluminum plant workers exposed to fluoride, serum SOD activity is enhanced.[10] The reason for this discrepancy is not known.

6.7 CHARACTERISTICS OF THE CYTOCHROME P450s

As mentioned earlier, cytochrome P450s consist of a number of isozymes. Researchers have divided the isozymes into several categories: CYP1, CYP2, and CYP3. These categories are further divided into subfamilies according to their properties. The nomenclature and major characteristics of the cytochrome P450 isozymes are presented in Table 6.1.

6.7.1 INDUCTION

As shown in Table 6.1, one of the characteristics of cytochrome P450s is that a number of the isozymes are inducible upon exposure to environmental

Table 6.1 Characteristics of the Cytochrome P450 Isozymes

Cytochrome P450 (CYP)	Subfamily	Characteristics
CYP1	CYP1A1	Found in human lung, skin, intestine, lymphocytes, and placenta (induced in the lungs of smokers)
	CYP1A2	Found in human liver; important in drug metabolism; thought to be responsible for metabolic activation of polycyclic aromatic hydrocarbons, aromatic amines, and nitrosamines
CYP2	CYP2D6	Found in human liver; important in drug metabolism; primarily metabolizes hydrophobic amines; possible link between rapid metabolizers and lung cancer
	CYP2E1	Found in human liver; inducible by ethanol, acetone, etc., important in metabolism of a large number of halogenated alkanes; involved in certain carcinogenesis activities, such as those involving nitrosamines, acrylonitrile, benzene, and vinyl chloride
CYP3	CYP3A4	Found in liver, small intestine, and kidney; inducible by glucocorticoids and phenobarbitol; important in drug metabolism; metabolizes a wide variety of hydrophobic substrates including activation of aflatoxin B_1, nitroaromatics, etc.
	CYP3A5	Found in placenta; expressed in liver in 15% of the population, but in 80% of all human kidneys; substrate specificity similar to CYP3A4
	CYP3A7	Found in fetal liver; not found in adults except in placenta; metabolizes dehydroepiandrosterone sulfate

chemicals or certain drugs. For instance, CYP1A1 and CYP1A2 are induced in smokers, while CYP2E1 is induced by ethanol and isoniazid, etc. Many studies have shown increases in MFO enzymes from organisms following exposure to xenobiotics. Such inducers of cytochrome P450 increase the rate of xenobiotic biotransformation. As a consequence of this phenomenon, it would be expected that there could be increases in the activation of procarcinogens to DNA-reactive metabolites, leading to increased tumor formation. However, it is not clear whether this is indeed the case in humans. In many instances, P450 induction does not necessarily enhance the biotransformation of the inducer. [6]

Fish from waters that receive pulp-mill effluents have been shown to respond to the effluents with increases in hepatic MFO activity, particularly that of EROD.[11] In a recent study, a compound isolated from a bleached-kraft mill effluent (tentatively identified as a chlorinated pterostilbene) was shown to be capable of causing MFO induction in rainbow trout and in a hepatocyte cell line.[12]

6.7.2 Genetic Polymorphisms

Another characteristic feature of cytochrome P450s is the occurrence of genetic polymorphism, resulting in enzyme levels and activities varying greatly between different individuals. Genetic polymorphism includes defects in CYP2D6 and variations in CYP1A2 activities. For example, three phenotypes are found in CYP1A2: slow, medium, and rapid metabolizers. Research shows that this phenomenon is related to susceptibility of individuals to certain types of cancer – for example, increased bladder and colorectal cancer susceptibility for rapid 1A2 populations. Similarly, marked ethnic differences exist with CYP2D6, and usually 1 to 10% of the population are poor metabolizers who may develop less-aggressive forms of bladder cancer. Conversely, a possible link appears to exist between rapid metabolizers and lung cancer.[13] Several P450s are also involved in steroid biosynthesis and metabolism. As mentioned previously, some researchers suggest that a general increase in estrogenic activity may be responsible for the observed increases in breast and testicular cancers.

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6.9 REVIEW QUESTIONS

1. What is the significance of biotransformation in the body's response to environmental chemicals?
2. What are the main differences between Phase I and Phase II reactions?
3. Give the three types of reactions in Phase I biotransformation.
4. List the names of the functional groups that participate in Phase I biotransformation.
5. List the characteristics of the mixed-function oxidases (MFO).
6. What specific role does the liver play in biotransformation?
7. List the endogenous substances that are associated with Phase II reactions.
8. What are the possible problems involved in biotransformation?
9. Give an overall reaction whereby an environmental chemical, RH, is oxidized by the cytochrome P450 system.
10. Name the four major antioxidant enzymes.
11. Give the names of cellular antioxidants that may prevent free-radical-mediated cellular damage.
12. Explain the important role that SOD plays in the cell.
13. Which environmental chemicals can inhibit microsomal enzymes?
14. List four environmental chemicals that can stimulate microsomal enzymes.
15. Briefly explain the term *genetic polymorphism*.
16. Explain how cytochrome P450s may be related to cancer.
17. What are the main differences between a substrate and the conjugate in biotransformation?

Chapter 7

Defense Responses to Toxicants

7.1 INTRODUCTION

As seen from the foregoing chapters, living organisms are subjected to the influence of a large number of environmental toxicants in addition to the essential nutrients that are absorbed. This chapter examines how organisms may be able to respond to the impact of many of those toxicants. The consequences that may result when such defense mechanisms fail will also be discussed.

7.2 RESPONSES OF HUMANS AND ANIMALS

This section focuses on five body systems, including the respiratory tract, gastrointestinal tract, membranes, liver, and kidneys in humans and, in some instances, in animals.

7.2.1 The Respiratory Tract

An adult breathes more than 13,000 liters of air a day. This is not only the body's largest intake of any substance but also the most immediately important to life. Humans can go without food for many days and without water for many hours without serious health effects, but life without air terminates in a very few minutes. Air is inhaled through the nasal cavity, nasopharynx, and trachea. The trachea divides into the main *bronchi*, which go to the right and left lungs (Figure 7.1). The right lung consists of three lobes, and the left lung, two. The bronchi divide into finer and finer tubes, called *bronchioles*. Located at the ends of the bronchioles are many tiny air sacs called *alveoli*, these are where the exchange of gases takes place. At the alveoli, a thin sheet of moving blood picks up molecular oxygen (O_2) from the inhaled air and unloads carbon dioxide (CO_2) for exhalation.

The respiratory tract is one of the principal ports of entry for air pollutants and is remarkably well equipped to cope with harmful invaders. There are three main processes that operate in their defense against the invasion of foreign agents: *filtration*, *inactivation*, and *removal*.

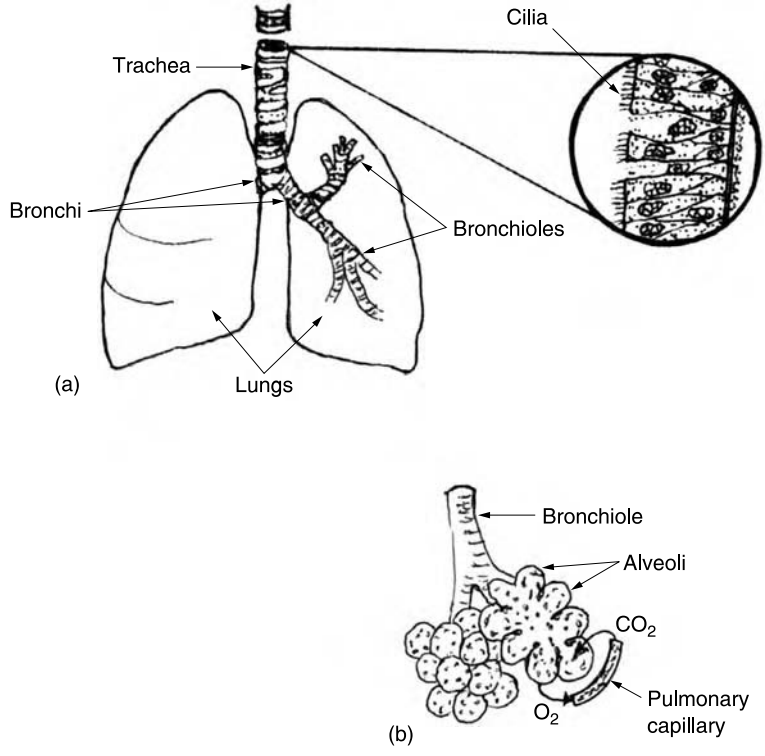

Figure 7.1 Generalized structure of human lungs: (a) the tracheobronchial area, with microscopic view showing a section of the ciliated epithelium that lines the passages (inset), and (b) alveoli.

7.2.1.1 Nasopharynx

Air that is drawn in through the nose and the upper throat is warmed and moistened as it moves to the lungs. Particulate matter is likewise moistened as it enters the nose. Large particles are filtered and removed by the hairs at the entrance of the nose, while smaller particulates, such as dust, carbon, and pollen spores, are washed out with the aid of mucus.

7.2.1.2 Tracheobronchial Areas

The response of the tracheobronchial area to large particulates is contraction of the muscles, causing the lumena of bronchi to be narrowed. This results in removal of solid particulate matter with a diameter above 5 mm, and permits less of the particulate matter to enter the lower portion of bronchial tubes. The mucus that is secreted moistens the particulates as they accumulate, which are then removed through the cough reflex. Spasm – involuntary muscular contraction – of the bronchi may be induced, which tends to prevent invading

agents from reaching the air sacs. However, this can also lead to respiratory distress.

A very important feature of the trachea is the action of cilia, hair-like structures that beat rhythmically back and forth in the air passage (Figure 7.1a). With a speed of 1300 beats per minute, billions of cilia function like a broom to sweep noxious foreign agents out of the system.

The condition commonly called bronchitis is caused by infection of the air passages, starting at the nose and extending through the bronchioles. Acute bronchitis may result from inhaled irritants, such as smoke, dust, and chemicals. It can also be due to allergy. Chronic bronchitis usually develops slowly and appears in people past the midway point of their lives. It occurs approximately four times more often in men than in women, and more often among city dwellers than rural residents. The most significant symptom is cough, which may be constant or intermittent. Mucus is almost always coughed up, which may be clear or may contain pus or streaks of blood. In many cases, because the patient is not severely ill or incapacitated, medical help is not sought, and so the cough and expectoration persist.

7.2.1.3 Alveoli

There are about 400 million alveoli in the lungs of a healthy adult. The inner surfaces of the alveoli, continuous with the bronchioles, bronchi, and trachea, are technically outside the body as they are in contact with the atmosphere. If the walls of all the air cells were spread out as one continuous area, they would cover a surface the size of a tennis court. Because this immense surface is compacted into the small space of two lungs, the walls of the air cells are extremely thin. This is essential to allow absorption of O_2 from air and dispersal of CO_2 waste gases to take place (Figure 7.1b). Particulate matter that reaches the alveoli and is deposited is usually 1μm or less in diameter. Particulates with a diameter less than 0.5 μm are small enough to behave like gases.

There are four types of cells in the alveoli: alveolar epithelial cells, endothelial cells, large alveolar cells, and alveolar macrophages. Alveolar epithelial cells are responsible for the exchange of CO_2 and O_2; alveolar endothelial cells are endowed with various protective properties; and large alveolar cells and alveolar macrophages carry out oxidative and synthetic processes that defend the lungs against invading organic and inorganic materials.

Macrophages play a well-known phagocytic role in the lungs and other tissues. They engulf an organism or a particle by membrane invagination and pouch formation, and are one of the most important components of the immune response. A number of environmental agents, such as silica, asbestos, cigarette smoke, carbon monoxide (CO), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), formaldehyde, and aflatoxin and other mycotoxins, can either depress or enhance the phagocytic function of macrophages.

The term *emphysema* derives from Greek words meaning "overinflated," the overinflated structures being alveoli. Tiny bronchioles through which air flows to and from the air sacs have muscle fibers in their walls. In an emphysematous patient, the structures of bronchioles and air sacs may become hypertrophied and lose elasticity. Air will flow into the air sacs easily but cannot flow out easily because of the narrowed diameter of bronchioles. The patient can breathe in but cannot breathe out efficiently, resulting in too much stale air in the lungs. As pressure builds up in the air cells, their thin walls are stretched to the point of rupture, so several air spaces communicate and the area of surfaces where gas exchange takes place is decreased. Figure 7.2 illustrates the comparison between a healthy person and an emphysematous patient in their alveoli and the volume of exhaled air.

Smog, smoke, and inhaled irritants may increase mucus secretion in the air passages and cause obstruction of bronchioles, with entrapment of air beyond the obstruction. The result is shortness of breath, overwork of the heart, and sometimes death. Some studies associate emphysema with smog, particularly NO_2 and ozone (O_3), SO_2, and heavy cigarette smoking.

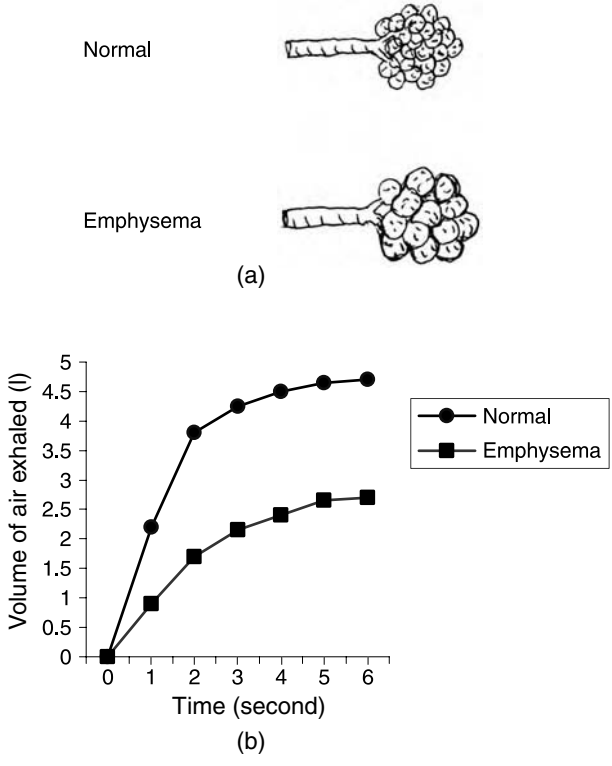

Figure 7.2 The effects of emphysema on lungs: (a) decrease in lung surface area due to overexpansion of alveoli, and (b) reduction in ability to exhale.

7.2.2 Gastrointestinal Tract

The small intestine, which comprises the duodenum, jejunum and ileum (Figure 7.3), is the main part of the gastrointestinal tract where nutrients from the diet are absorbed into the bloodstream. A toxic agent may be absorbed into the bloodstream through the same route. The villi, 0.5 to 1 mm long structures that line the small intestine, contain lymphoid capillary surrounded by a network of blood capillaries. The villi, and the smaller microvilli, can readily take up both nutrients and any toxic agents present in our diet. Mechanisms involved in the removal of noxious agents from the gastrointestinal tract include spastic movements in the stomach and bowels, leading to vomiting and speedy propulsion of fecal matter through the entire intestinal tract.

Readily soluble toxicants may be promptly absorbed into the bloodstream, whereas less soluble chemical agents are carried into the lower portion of the bowels and eliminated with feces. Small particles, up to 50 μm in size, can penetrate the intestinal wall between epithelial cells and be transported through lymphatic system and blood vessels to the liver and other organs.

In passing through the intestinal tract a toxic agent may induce diarrhea and spastic pains or constipation. Mucus and blood may often be observed in the stool. If the poisoning extends over long periods, chronic changes occur. Metals, such as lead (Pb) and mercury (Hg), and arsenic (As) and fluoride are known to induce chronic illness. Interference with the normal function of the

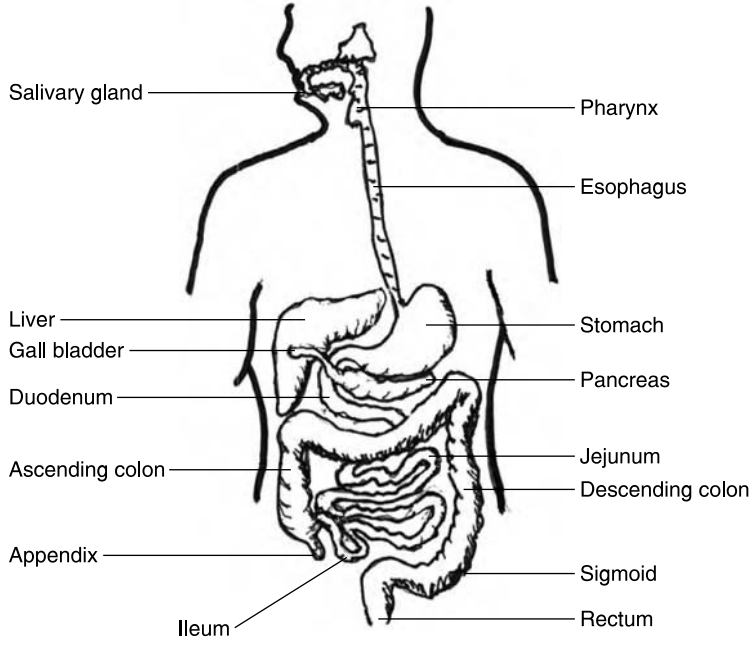

Figure 7.3 The human digestive system.

lower bowels by toxic agents leads to loss of water, sodium (Na), and other vital minerals and vitamins.

7.2.3 Membranes

The plasma and intracellular membranes of mammalian cells have similar overall compositions: about 60% protein and 40% lipid by weight. In addition, some membranes also contain small amounts of carbohydrate, as glycoproteins or glycolipids. The human erythrocyte membrane, for example, contains approximately 10% carbohydrate, which appears to be localized on the outer surface of the membranes.

The overall arrangement of the protein and lipid components in a typical membrane is illustrated in Figure 7.4. It is clear that the basic structural feature is a phospholipid bilayer with embedded protein complexes. This characteristic structure enables the permeability of the cell barrier. Phospholipids are the major structural components of lipid bilayers. They consist of mainly phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin, and phosphatidyl serine. The other major lipid is cholesterol. All phospholipids are composed of two hydrophobic hydrocarbon chains, linked to a charged polar headgroup via the glycerol backbone. Phospholipid bilayer membranes therefore consist of a hydrophobic core, largely impermeable to water and other hydrophilic solutes, with polar surfaces that may or may not bear a net surface charge depending on the particular phospholipids. Membrane proteins are grouped into two categories: extrinsic proteins and intrinsic proteins. Some of the membrane proteins are structural but others are enzyme proteins such as ATPase and cytochrome oxidase.

The cell membrane serves as the major barrier to the absorption of toxic foreign compounds. The membranes may be those surrounding the cells of the skin, the cells lining the gastrointestinal tract or those of the alveoli in the lung. The passage of a compound across one of these membranes is therefore an

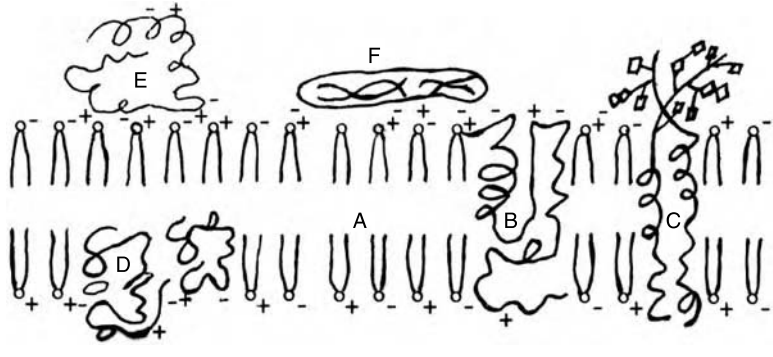

Figure 7.4 Arrangement of protein, lipid, and carbohydrate components in biological membranes. A = lipid bilayer region; B–D = intrinsic proteins, e.g., cytochrome oxidase (B), glycophorin with sugar residues indicated (C), cytochrome b (D); E, F = extrinsic proteins, e.g., cytochrome c.

important factor in absorption. In addition, membranous barriers influence translocation of any chemical from the exterior of a cell to the intracellular fluid of a cell within an animal. A toxicant that gains entry by the mouth must pass from the gastrointestinal tract to the circulation and then to the cell. Such a process involves a series of translocation steps and increases the possibility of exposure of the chemical to large endogenous molecules, such as proteins, which may effectively bind and therefore functionally change and remove the offending chemical.

Certain chemicals, however, may react with membrane material, such as proteins, thus altering the membrane structure. For example, heavy metals such as Pb, cadmium (Cd), and Hg may react with the –SH groups on membrane protein molecules. Similarly, the lipid constituent of the membrane may be altered by peroxidation by O_3, as mentioned previously. Free radicals formed in the reaction may attack not only lipids but also proteins, leading to disruption of the membrane.

7.2.4 Liver

The liver, the largest solid organ of the body (Figure 7.3), is an incomparable chemical plant. As discussed in Chapter 4, the liver plays the foremost role in detoxifying xenobiotics. In addition, it is a blood reservoir and a storage organ for some vitamins, and for digested carbohydrate (as glycogen), which is broken down releasing glucose to sustain blood sugar levels. The liver is also a manufacturing site for enzymes, cholesterol, proteins, vitamin A (from carotenoids), blood coagulation factors, and other molecules.

Although the liver is noted for its ability to regenerate (under certain conditions), it can nevertheless be severely damaged. For example, *cirrhosis* (a chronic progressive disease of the liver that is characterized by an excessive formation of connective tissue, followed by hardening and contraction), which is related to alcoholism and poor nutrition, may be caused by chronic exposure to chemicals such as carbon tetrachloride (CCl_4). Another liver disease is *fibrosis*, characterized by the deposition of excessive amounts of collagen such that the features of the hepatic lobules are accented. Hepatic fibrosis can result from repeated exposure or continuous injury following prolonged low-level exposure to environmental chemicals. Portal fibrosis with portal hypertension has also been reported in humans repeatedly exposed to As[1] compounds or vinyl chloride.[2,3]

7.2.5 Kidneys

The kidneys (Figure 7.5) are the principal organs for excretion of both endogenous and exogenous toxins. Approximately one fourth of the blood pumped by each beat of the heart passes through the kidneys. The kidneys incessantly filter various substances from the blood, reabsorb some of them, and concentrate wastes created by metabolic processes in urine. Optimal mechanisms for excretion depend on selective conservation of essential

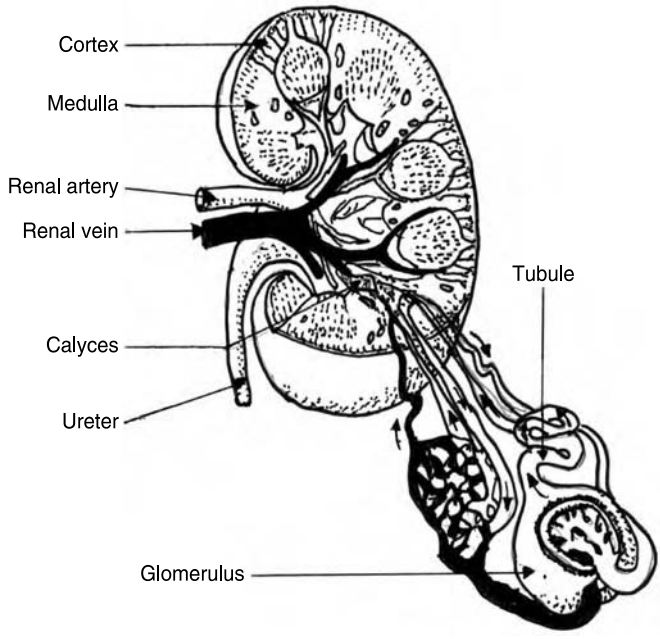

Figure 7.5 The structure of the human kidney.

nutrients and their metabolites, as well as upon transport of toxins, so reducing the potential for cell injury. The urine-forming unit of the kidney is called a nephron. It is a microscopic filtration structure consisting of several intricate substructures, including the Bowman's capsule and the glomerulus. The glomerulus (meaning "little ball"), a tufted network of intricately laced capillaries, is nested in the capsule and terminates in a collecting tubule located towards the central part of the kidney. Practically all the constituents of blood, except blood cells and most proteins, can pass from the capillaries into the space between the double walls of the capsule. The resulting filtrate contains many dissolved materials, some of which are indispensable for the body's functioning, while some others may be harmful.

The filtering process of the glomeruli is physical, not chemical. The area of the filtering surface of glomeruli of a single kidney is as large as the surface of the entire body, and the glomerular capillaries of both kidneys would stretch more than 35 m if laid end to end. The filtrate is very dilute, and is mostly water. Out of some 200 l of filtrate a day, an average adult concentrates about 1.5 l of urine. It is obviously essential that most of the filtrate and many of its dissolved materials be reabsorbed, while only harmful materials are excreted. This is a function of the kidney tubules (Figure 7.5), in which residues are gradually concentrated into urine.

Generally, the ability of the glomerular capillary wall to filter macromolecules is inversely proportional to the molecular weight of a substance:

small molecules are freely filtered, while large molecules, such as certain proteins, are restricted. Filtration of anionic molecules is likewise more restricted than filtration of neutral or cationic molecules of the same size. Toxicants that neutralize or decrease the number of fixed anionic charges on glomerular structural elements will impair the charge- or size-selective properties of the glomerulus, leading to urinary excretion of polyanionic or high-molecular-weight proteins.[4]

Environmental chemicals, including metals and drugs, may be transported across proximal tubular cells, i.e., from renal capillaries across tubular cells to be excreted in tubular lumena or vice versa. Many cationic substances are excreted against concentration gradients at rates greater than the glomerular filtration rate. This indicates an active-transport process. Such a process requires expenditure of energy derived from oxidative metabolism carried out in mitochondria. However, active transport that has the capability of concentrating absorbed material may concentrate potential nephrotoxins as well as essential substances in the renal cortex. The same toxins that cause adverse effects on energy metabolism will impede the cellular transport of essential solutes. Other toxic substances may also be concentrated in the medulla.

As noted previously, metabolism of chemicals within the kidney may produce substances that are either more or less toxic than the parent chemical. For instance, trichloromethane ($CHCl_3$) and CCl_4 may be biotransformed into reactive, toxic products that bind covalently to renal tissue, leading to membrane injury. Exposure to certain other substances may result in activation or enhancement of enzyme systems, such as the mixed-function oxidase (MFO). The toxicity of methoxyfluorane, for example, may be enhanced as a result of increased metabolism, as the metabolic products, i.e., fluoride and oxalate, are both known to be potentially toxic to the kidney. Fluoride ions are toxic to cell membranes, whereas oxalate may accumulate within the lumena of nephrons.

Heavy metals, such as Pb, Cd, and Hg, are known also to cause renal disease. The adverse effects of Pb may be both acute and chronic. Cells of the proximal tubules are most severely affected, as shown by reduction in resorptive function of nutrients such as glucose and amino acids. Conversely, the effect of inorganic Cd salts on the kidney is largely chronic. The characteristics of Cd nephropathy include increased Cd in the urine, proteinuria, aminoaciduria, glucosuria, and decreased renal tubular reabsorption of phosphate. With chronic exposure to toxic levels, renal tubular acidosis, hypercalciuria, and calculi formation occur.[5]

Hg is known to produce different effects on kidneys, depending on the biochemical form of the metal and nature of exposure. Inorganic Hg compounds can cause acute tubular necrosis, whereas chronic low-dose exposure to mercuric salts or elemental Hg vapor may induce an immunologic glomerular disease. The presence of proteins rich in cysteine may be able to alleviate Hg toxicity. As noted in Chapter 5, Se is known to antagonize Hg, reducing its toxicity.

An interesting phenomenon concerning the toxicity of Cd is the role that metallothionein (MT) plays. MTs are low-molecular-weight, nonenzymatic proteins that are ubiquitous in the animal kingdom. They have a unique composition as they do not contain aromatic amino acids, but are rich in cysteine (which consists of one third of the amino acid residues), and are therefore capable of binding metals such as Zn and Cd. Various physiologic and toxicologic stimuli can induce MT genes. The formation of MTs following exposure to Cd appears to protect the body against Cd toxicity.[6]

The mammalian kidney is unusually susceptible to the toxic effects of various noxious chemicals. This is attributed, in part, to the unique physiologic and anatomical features of the kidney. The kidneys receive 20 to 25% of the resting cardiac output, even though they make up only about 0.5% of total body mass. Therefore, relatively high amounts of any chemical or drug in the systemic circulation will be delivered to the kidneys. As kidneys form concentrated urine, they also tend to concentrate potential toxicants in the tubular fluid. Therefore, a toxicant present at nontoxic levels in the plasma may reach toxic levels in the kidney. Moreover, as noted previously, kidneys are involved in renal transport, accumulation, and metabolism of xenobiotics. As kidneys participate in these processes, they will clearly increase their susceptibility to toxic injury.[4]

7.3 RESPONSES OF PLANTS

Chapter 5 described several physiological and biochemical mechanisms that exist in plants that may protect them against the toxic effects of pollutants absorbed into the tissue. For example, the sensitivity of onion plants to O_3 was found to vary between different cultivars. Following exposure to O_3, the stomata of the resistant cultivar were closed with no appreciable injury, whereas the stomata of the sensitive cultivar remained open, with obvious injury.[7]

The study of phytochelatins in plants has attracted recent attention. Studies have shown that plants exposed to heavy metals, particularly Cd or Pb, produce phytochelatins. Phytochelatins are sulfur-rich polypeptides that occur in plants, with function similar to that of mammalian MT discussed above. The general structure of phytochelatins is $(–Glu–Cys)_n–$ Gly, where n is 2 to11. The –SH group contained in cysteine can bind covalently to heavy metals, as discussed in Section 4.4.3.2.

The occurrence and free-radical scavenging action of cellular antioxidants are discussed in Chapter 6. Various free radicals are formed naturally in cellular metabolism. Endogenous antioxidants (such as vitamins E and C and glutathione (GSH)) and antioxidant enzymes (including superoxide dismutase (SOD), catalase, glutathione peroxidase, and GSH reductase) help detoxify the free radicals. Laboratory studies have shown that the activity of SOD is enhanced in tissues exposed to low concentrations of sodium fluoride (NaF),

while after exposure to high concentrations of NaF, SOD activity was depressed.[8,9]

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7.5 REVIEW QUESTIONS

1. What is acute bronchitis? How does it occur?
2. How does chronic bronchitis occur?
3. What is the function of alveoli?
4. Which of the following types of cells are responsible for the exchange of CO_2 and O_2? (a) alveolar epithelial cells, (b) endothelial cells, (c) large alveolar cells, (d) alveolar macrophages.
5. What is emphysema? Briefly explain how it occurs.
6. What is the function of a macrophage, and how does it perform its function?
7. Which environmental agents can affect the function of macrophages?
8. What is the composition of the membranes of mammalian cells?
9. Explain the characteristics of phospholipid bilayer in membranes.
10. What is metallothionein (MT)? What is unique about the amino acid composition of MTs?
11. Explain how MTs are related to Cd exposure.

12. Why are the kidneys susceptible to toxic injury?
13. What are phytochelatins? What is the function of phytochelatins?
14. What are the compositional characteristics of phytochelatins?
15. How do heavy metals such as Pb and Cd damage membranes?

Chapter 8

Air Pollution – Inorganic Gases

8.1 INTRODUCTION

This chapter considers four of the major gaseous air pollutants: sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3), and carbon monoxide (CO). The importance of these gaseous air pollutants is emphasized by the fact that they are four of the six "Criteria Air Pollutants" regulated by the U.S. Environmental Protection Agency (EPA). The other two criteria air pollutants are volatile organic compounds (VOCs) and lead (Pb). VOCs are discussed in Chapter 11, while Pb is included in Chapter 12.

8.2 SULFUR DIOXIDE

SO_2 and sulfur trioxide (SO_3) are the two sulfur oxides (SO_x) that are important air pollutants. This chapter focuses on SO_2 because it is far more important than SO_3 as an air pollutant. In fact, based on the quantities emitted into the atmosphere, SO_2 is considered the most dangerous of all gaseous pollutants.

8.2.1 Sources of SO_2

Atmospheric SO_2 arises from both natural and anthropogenic sources. Sulfur compounds are emitted naturally through volcanic action, sea salt over the oceans, and decomposition of organic matter (mostly as hydrogen sulfide, H_2S). Most anthropogenic emissions of sulfur (S) to the atmosphere (about 95%) are in the form of SO_2. The main human activities that cause SO_2 emission include combustion of coal and petroleum products, petroleum refining, and nonferrous smelting. In the U.S., about 95% of the total emission is from industry and stationary sources.

The S content of coal ranges from 0.3 to 7%, and it is present in both organic and inorganic forms, whereas in oil the content ranges from 0.2 to 1.7%, and the S is in organic form. The most important S-containing compound in coal is iron disulfide or pyrite (FeS_2). When heated to high temperatures, pyrite is oxidized through the reactions shown below:

$$FeS_2 + 3O_2 \rightarrow FeSO_4 + SO_2 \tag{8.1}$$

$$4FeS_2 + 11O_2 \rightarrow 2Fe_2O_3 + 8SO_2 \tag{8.2}$$

In the smelting process, sulfide ores of copper (Cu), Pb, and zinc (Zn) are oxidized (roasted), forming metallic oxides. For example, zinc sulfide (ZnS) is converted in a smelter to zinc oxide (ZnO), releasing SO_2:

$$2ZnS + 3O_2 \rightarrow 2ZnO + 2SO_2 \tag{8.3}$$

8.2.2 Characteristics of SO_2

SO_2 is highly soluble in water (solubility: 11.3 g per 100 ml). When SO_2 is emitted into the atmosphere, it can dissolve in fog or cloud droplets, forming sulfurous acid (H_2SO_3), which is readily oxidized by molecular oxygen (O_2) to sulphuric acid (H_2SO_4). The formation of H_2SO_4 by this process is greatly facilitated by some metal salts, which are also dissolved in the droplets. Any ammonia (NH_3) present in the atmosphere will rapidly react with the H_2SO_3 or H_2SO_4 droplets to form ammonium sulfate or ammonium bisulfate.[1]

Atmospheric SO_2 may be removed by several competing processes: direct removal by deposition as bisulfate in precipitation, incorporation into fog and cloud droplets (where it is oxidized catalytically and photochemically to sulfate), or diffusion to plant surfaces where it is adsorbed and reacts chemically. According to Fox,[2] both dry and wet forms of H_2SO_4 produced in the atmosphere may be removed by deposition to the earth's surface.

Studies show that the photochemistry of the free hydroxyl radical ($OH^{\cdot}$) controls the rate at which many trace gases, including SO_2, are oxidized and removed from the atmosphere.[3] The photochemistry involving the $OH^{\cdot}$ radical is shown in Figure 8.1.

8.2.3 Effects on Plants

SO_2 enters plant leaves predominantly by gaseous diffusion through stomatal pores, as do other atmospheric pollutants. The number of stomata and the size of aperture are important factors affecting SO_2 uptake. Other factors, such as light, humidity, temperature, and wind velocity, are also important because they influence the turgidity of stomatal guard cells. Low concentrations of SO_2 can injure epidermal and guard cells, resulting in elevated stomatal conductance and greater entry of SO_2 into plants.

Following uptake by plant leaves, SO_2 is rapidly translocated through the plant. It can then affect photosynthesis, transpiration, and respiration, the three major functions of plant leaves. A slight increase in both net photosynthesis and transpiration may occur at low SO_2 concentrations for short periods, followed by a decrease in both processes. Higher SO_2 concentrations induce immediate decreases in these processes. Plant injuries may be manifested by leaf chlorosis and spotty necrotic lesions (Figure 8.2). As noted previously (Table 5.1), a synergistic effect on leaf damage occurs when plants are exposed to SO_2 and O_3 simultaneously. Damage to mesophyll cells commonly occurs, which is the main cause of observed changes in photo-

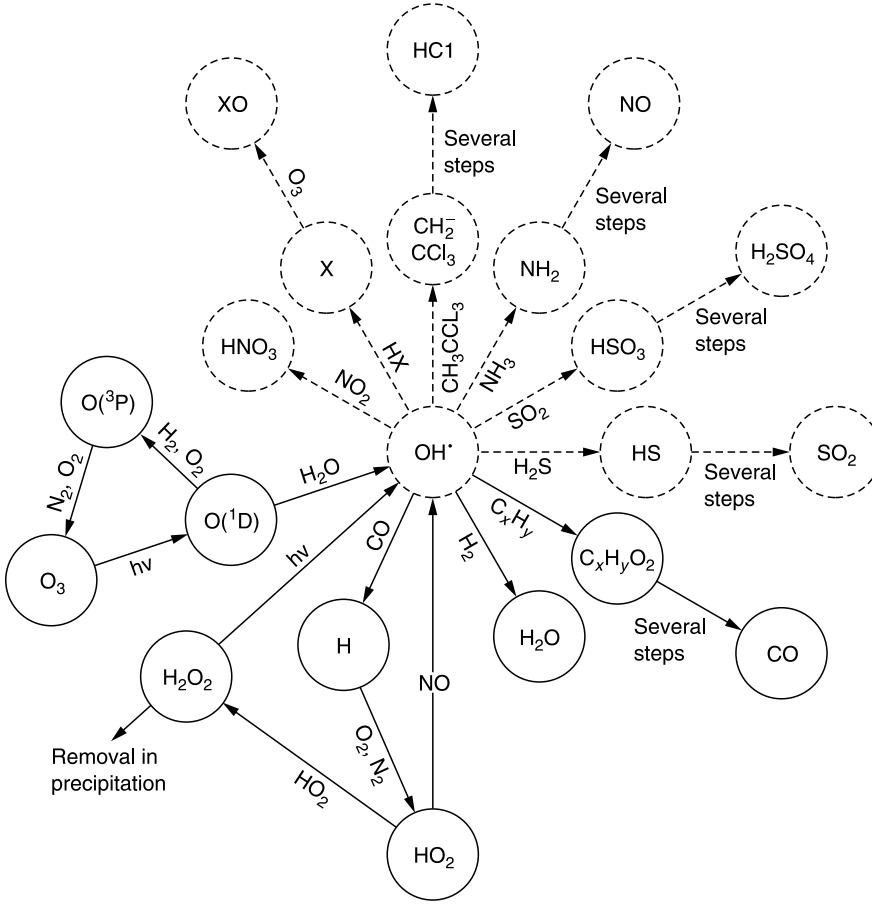

Figure 8.1 The photochemistry of the free hydroxyl radical, $OH^{\cdot}$, controls the rate at which many trace gases are oxidized and removed from the atmosphere. Processes that are of primary importance in controlling the concentration of $OH^{\cdot}$ in the troposphere are indicated by a solid line; those that have a negligible effect on $OH^{\cdot}$ levels but are important because they control the concentrations of associated reactions and products are indicated by a broken line. Circles indicate reservoirs of species in the atmosphere; arrows indicate reactions that convert one species to another, with the reactant or photon needed for each reaction indicated along each arrow. Multistep reactions actually consist of two or more sequential elementary reactions. HX = HCl, HBr, HI, or HF. C_xH_y denotes hydrocarbons.
Source: adapted from W.L. Chameides and D.D.Davis, *C&E News*, Oct. 4, 1982. With permission from American Chemical Society.

synthesis. Exposure of Chinese guger-tree seedlings grown in field chambers with 325 ppb of SO_2 for 4 weeks showed rapid decreases in photosynthetic rate, root weight, and total seedling weight.[4] A simultaneous increase (75%) in –SH groups in leaves was observed.

Once absorbed into a leaf, SO_2 readily dissolves in the intercellular water to form bisulfite (HSO_3^-), sulfite (SO_3^{2-}), and other ionic species (Figure 8.3).

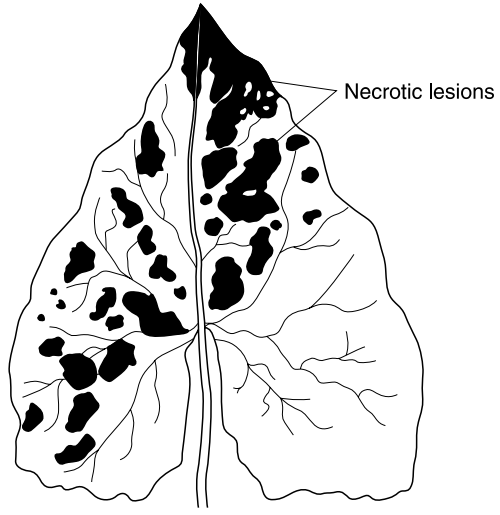

Figure 8.2 Leaf damage induced by SO_2.

Both SO_3^{2-} and HSO_3^- have a lone pair of electrons on the S atom that strongly favors reactions with electron-deficient sites in other molecules. They are both phytotoxic, affecting several physiological and biochemical processes of plants.[5] The phytotoxicity of SO_3^{2-} and HSO_3^- is diminished when these species are converted to less toxic forms, such as SO_4^{2-}. For instance, oxidation of HSO_3^- to SO_4^{2-} can occur both enzymatically and non-enzymatically. Several factors, including cellular enzymes such as peroxidase and cytochrome oxidase, metals, ultraviolet (UV) light, and superoxide ($O_2^{\cdot -}$),

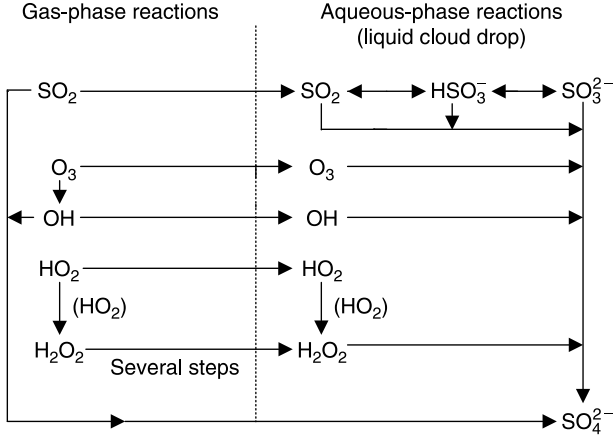

Figure 8.3 Fate of SO2 in tissues. Arrows crossing liquid cloud drop barrier signify heterogeneous reactions that transfer a species from the gas phase to the aqueous phase. *Source*: adapted from Chameides, W. L. and Davis, D. D, *C&E News*, Oct. 4, 1982. With permission from American Chemical Society.

stimulate the oxidation of SO_2. In the presence of SO_3^{2-} and HSO_3^-, more $O_2^{\cdot -}$ is formed by free-radical chain oxidation. Other free radicals may also be formed. These oxidizing radicals can have detrimental effects on leaf cells. Alternatively, SO_3^{2-} and SO_4^{2-} formed may be reduced and assimilated with a carbon skeleton to cysteine.[6]

Plant metabolism has been shown to be affected by SO_2 in a variety of ways: stimulation of phosphorus (P) metabolism and reduction in foliar chlorophyll concentration,[7] increase or decrease in carbohydrate concentrations in red kidney bean plants exposed to low or high levels of SO_2,[8] and inhibition of lipid biosynthesis in pine needles treated with SO_2.[9]

Malhotra and Khan[9] found that pine-needle tissues, particularly the developing tissues, actively incorporate acetate [1-^{14}C] into phosphogalacto- and neutral lipids. The major incorporation of the label among these lipids was always in the phosphatidyl choline fraction. Treatment of needle tissues with gaseous or aqueous SO_2 markedly inhibited lipid biosynthesis. A partial or complete recovery in lipid biosynthesis capacity occurred when plants were removed from the SO_2 environment.

SO_2 has been shown to affect a number of enzyme systems in different plant species. Enzymes studied include alanine and aspartate aminotransferases, glutamate dehydrogenase, malate dehydrogenase, glycolate oxidase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, fructose-1,6-bisphosphatase, ribulose-5-phosphate kinase, peroxidase, and superoxide dismutase (SOD). Enzyme activity may be enhanced or depressed by exposure to SO_2 at different concentrations. With Chinese guger-tree seedlings exposed to 325 ppb of SO_2, for example, peroxidase activity increased significantly, while SOD activity was unaffected.[4]

It is widely known that differences in tolerance of plant species to SO_2 occur under similar biophysical conditions. This suggests that delicate biochemical and physiological differences in plants could affect the sensitivity of a particular plant species to SO_2.

8.2.4 Effects on Animals

Although SO_2 is an irritating gas for the eyes and upper respiratory tract, no major injury from exposure to any reasonable concentrations of this gas has been demonstrated in animal experiments. Even exposure to pure gaseous SO_2 at concentrations 50 or more times ambient values produced little distress.[10,11] Concentrations of 100 or more times ambient are required to kill small animals. Mortality is associated with lung congestion and hemorrhage, pulmonary edema, thickening of the interalveolar septa, and other relatively nonspecific changes of the lungs, such as pulmonary hemorrhage and hyperinflation. These changes were associated with salivation, lacrimation, and rapid, shallow ventilation. Mice exposed to 10 ppm SO_2 for 72 hours showed necrosis and sloughing of the nasal epithelium.[12] The lesions were more severe in animals with preexisting infection. Other symptoms include decreased

weight gains, loss of hair, nephrosis in kidneys, myocardial degeneration, and accelerated aging.

Many studies have demonstrated the health effects of acidic aerosols on laboratory animals. Changes in pulmonary function, particularly increases in pulmonary flow resistance, occur after acute exposure. H_2SO_4 is shown to be more irritating than any of the sulfate salts in this regard. The irritant effect of H_2SO_4 depends in part on droplet size, smaller droplets being more effective.[13] For instance, animals exposed to 0.3 to 0.6 μm H_2SO_4 droplets at various concentrations showed either slowed or accelerated bronchial mucociliary clearance function, depending on the concentration of the aerosol. Studies on the comparative effects of exposure to H_2SO_4 and ammonium bisulfate (NH_4HSO_4) showed alteration of phagocytic activity, with more pronounced effect exhibited by H_2SO_4. Repeated exposures to H_2SO_4 caused the production of hyper-responsive airways in previously healthy animals. Such exposure also resulted in histological changes, such as increased numbers of secretory cells in distal airways and thickened epithelium in airways of midsized bronchi and terminal bronchioles.[14]

8.2.5 Health Effects

Epidemiological evidence from studies during the London smog episodes suggests that effects of SO_2 may occur at or above 0.19 ppm (24-hour average), in combination with elevated particulates levels. Short-term, reversible declines in lung function may occur at SO_2 levels above 0.10 to 0.18 ppm. These effects may be caused by SO_2 alone, or by formation of H_2SO_4 or other irritant aerosols. It appears more likely that the role of SO_2 involves transformation products, such as acidic fine particles. H_2SO_4 and sulfates have been shown to influence both sensory and respiratory function, such as increased respiratory rates and tidal volumes, and slowing of mucus clearance in humans.[15]

The effect of SO_2 on human health varies markedly with the health status and physical activity of individuals. For example, in asthmatics and others with hyper-reactive airways exposed to SO_2 at 0.25 to 0.50 ppm and higher while exercising, rapid bronchoconstriction (airway narrowing) was shown as the most striking acute response. This is usually demonstrated by elevated airway resistance, lowered expiratory flow rates, and the manifestation of symptoms such as wheezing and shortness of breath. The time required for SO_2 exposure to induce significant bronchoconstriction in exercising asthmatics is brief. Exposure durations as short as 2 minutes at 1.0 ppm have produced significant responses.[16] The combined effect of SO_2 and cold, dry air exacerbates the asthmatic response.[17] The bronchoconstrictive effects of SO_2 are reduced under warm, humid conditions.[18]

Exposure to submicrometer-sized H_2SO_4 aerosols increases tracheobronchial and alveolar rates of clearance in humans, the effects increasing with in line with SO_2 concentration and duration. Although the altered clearance rates may be an adaptive response of the mucociliary system to acid exposures, they may also be early stages in the progression toward more serious dysfunctions,

such as chronic bronchitis. Many researchers consider that chronic bronchitis in exposed persons may result from continued irritant exposures. In asthmatics, inhalation of acidic aerosols may lead to bronchospasm. Certain morphological changes are associated with the observed clinical symptoms in human chronic bronchitis. The changes include an increase in the number and size of epithelial mucus secretory cells, or both, in both proximal bronchi and in peripheral airways. The changes are accompanied by an increase in the volume of mucus secretion.[19] These changes are followed by an increase in epithelial thickness and a decrease in airway diameter, similar to those observed in laboratory animals.

Synergism may be observed in elevated airway resistance induced by SO_2 in combination with certain other air pollutants. For example, the response to inhaled SO_2 can be exacerbated by prior exposure to O_3. Also, the presence of H_2SO_4 on ultrafine ZnO particles (simulating coal combustion effluent) in a mixture with SO_2 has been shown to increase lung reactivity responses by ten-fold over those produced by pure droplets of H_2SO_4 of comparable size.[20]

Published reports support the hypothesis that acidic pollutants contribute to carcinogenesis in humans. Researchers have also examined possible biological mechanisms for such a contribution, including pH modulation of toxicity of xenobiotics and pH-dependent alteration of cells involving mitotic and enzyme regulation. Based on review of the mortality data from London for the period 1958 to 1972, the EPA[21] concluded that marked increases in mortality occurred, mainly among the elderly and chronically ill, and that the increases were associated with black smoke and SO_2 concentrations above 1000 $\mu g/m^3$. The conclusion was especially favored when such an elevation of pollutants occurred for several consecutive days.

8.3 NITROGEN DIOXIDE

8.3.1 Forms and Formation of Nitrogen Oxides

Six forms of nitrogen (N) oxides occur in the atmosphere: nitrous oxide (N_2O), nitric oxide (NO), nitrogen dioxide (NO_2), nitrogen trioxide (N_2O_3), nitrogen tetroxide (N_2O_4), and nitrogen pentoxide (N_2O_5). Of these, NO_2 is the most important air pollutant because of its relatively high toxicity and its ubiquity in ambient air, while N_2O, N_2O_3, and N_2O_4 have low relative toxicity and air pollution significance. Basic chemical reactions involved in NO_2 formation are as below:

$$N_2 + O_2 \xrightarrow{1210^\circ C} 2NO \tag{8.4}$$

$$2NO + O_2 \rightarrow 2NO_2 \tag{8.5}$$

The NO formed in Reaction 8.4 persists when temperature is cooled rapidly, as is the case in ambient air. Reaction 8.5 is one of the few that are slowed down by an increase in temperature.

8.3.2 Major Reactive N Species in the Troposphere

Several reactive N species, including NO, NO_2, nitric acid (HNO_3), occur in the troposphere. Among these, NO_2 is of particular environmental concern because it plays a complex and important role in the production of photochemical oxidants and acidic deposition. NO_2 is a unique air pollutant because it absorbs UV light energy and is then broken down to NO and atomic oxygen. The energetic oxygen atom reacts with molecular oxygen to form O_3. The resultant O_3 then reacts with NO to form molecular oxygen and NO_2, thus terminating the photolytic cycle of NO_2 (Figure 8.4). It is clear from Figure 8.4 that, as far as the cycle is concerned, there is no net gain or loss of chemical substances. However, accumulation of O_3 does occur (for reasons that will be discussed in the Section 8.4.1) and with numerous other photochemical reactions occurring in the troposphere, production of photochemical smog ensues.

In addition to NO and NO_2, HNO_3 (nitric acid) is another important N compound in the troposphere. Although HNO_3 is produced mainly from the reaction between NO_2 and $OH^{\cdot}$, it is formed through a secondary reactive pathway as well. In this case, NO_2 is first oxidized to NO_3 by O_3. The resultant NO_3 reacts with a molecule of NO_2, producing N_2O_5. The N_2O_5 combines with a molecule of water, yielding HNO_3. HNO_3, in turn, may be precipitated through rainout or dry deposition (Figure 8.5).

8.3.3 Effects on Plants

Plants absorb gaseous NO_x through stomata. NO_2 is more rapidly absorbed than NO, mainly because of its rapid reaction with water (NO is almost insoluble in an aqueous medium). The absorbed NO_2 is converted to nitrate

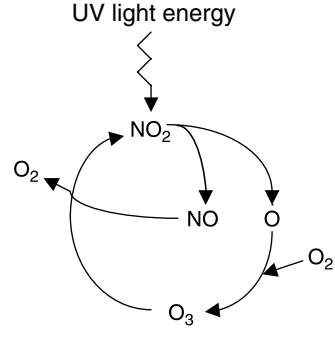

Figure 8.4 The photolytic cycle of NO_2.

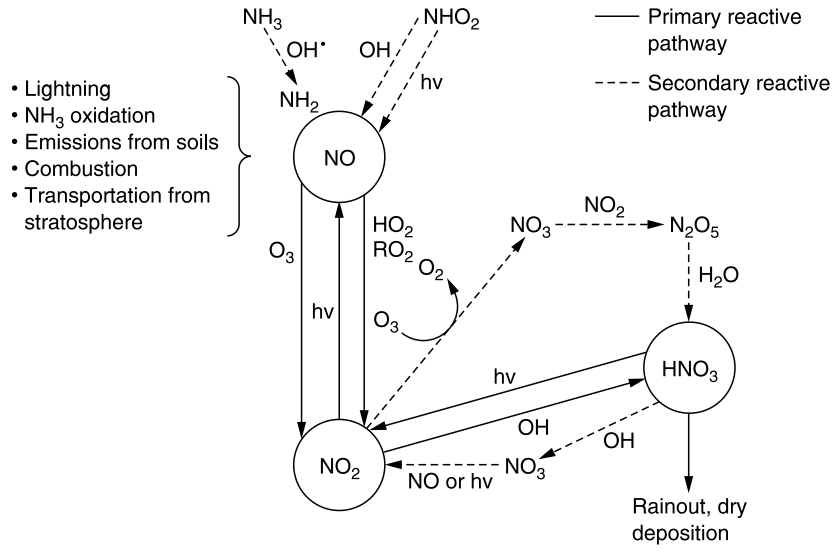

Figure 8.5 Major reactive N species in the troposphere.
Source: adapted from Chameides, W. L. and Davis, D. D, *C&E News*, Oct. 4, 1982. With permission from American Chemical Society.

(NO_3^-) and nitrite (NO_2^-) ions before the plant can metabolize it. NO_2-induced plant injury may be due to either acidification or a photooxidation process.[22] Symptoms exhibited by plants exposed to NO_2 are similar to those observed in plants exposed to SO_2, but much higher concentrations are required to cause acute injury. However, decreased photosynthesis has been demonstrated even at concentrations that do not produce visible injury. The combined effect of NO and NO_2 gases appears to be additive.

Photosynthetic inhibition caused by NO_x may be due to competition for NADPH between the processes of nitrite reduction and carbon assimilation in chloroplasts. NO_2 has been shown to cause swelling of chloroplast membranes.[23] Biochemical and membrane injuries may be caused by NH_3 produced from NO_3^-, if NH_3 is not utilized soon after its formation. Plants can metabolize the dissolved NO_x through their NO_2 assimilation pathway, as shown below:

$$NO_x \rightarrow NO_3^- \rightarrow NO_2^- \rightarrow NH_3 \rightarrow \text{amino acids} \rightarrow \text{proteins}$$

Other biochemical pathways affected by NO_x include inhibition of lipid biosynthesis, oxidation of unsaturated fatty acids *in vivo*, and stimulation of peroxidase activity.

8.3.4 Health Effects

Studies on the pathological and physiological effects of NO_2 on animals have been conducted at concentrations much higher than those found in ambient

air. The toxic action of NO_2 is mainly on the deep lung and peripheral airway. In various species of animals studied, exposure to NO_2 at 10 to 25 ppm for 24 hours was shown to induce the production of fibrin in the airway, an increased number of macrophages, and altered appearance of the cells in the distal airway and adjacent pulmonary alveoli. Terminal bronchioles showed hyperplasia and hypertrophy, loss of cilia, and disturbed ciliagenesis. Large crystaloid depositions also occurred in the cuboidal cells. Continuous exposure for several months produced thickening of the basement membranes, resulting in narrowing and fibrosis of the bronchioles. Emphysema-like alterations of the lungs developed, followed by death of the animals.[24]

As mentioned previously, although almost all the studies reported were conducted by using much higher concentrations of NO_2 than are found in ambient air, a few studies have dealt with low NO_2 concentrations. Orehek et al.[25] showed that asthmatic subjects exposed to 0.1 ppm of NO_2 resulted in significantly aggravated hyper-reactivity in the airway. While the health effects of prevailing concentrations of NO_2 are generally considered insignificant, NO_2 pollution may be an important aspect of indoor pollution. Evidence suggests that gas cooking and heating of homes, when not well vented, can increase NO_2 exposure and that such exposure may cause increased respiratory problems among individuals, particularly young children.

NO_2 is highly reactive and has been reported to cause bronchitis and pneumonia, as well as to increase susceptibility to respiratory infections (Table 8.1).[26] Epidemiological studies suggest that children exposed to NO_2 are at higher risk of respiratory illness. NO_2 exposure has been shown to impair immune responses, and be associated with daily mortality in children less than five years old, as well as with intrauterine mortality levels in Sao Paulo, Brazil.[27]

8.3.5 Biological Effects

Inhaled NO_2 is rapidly converted to NO_2^- and NO_3^- ions in the lungs, and these ions will be found in the blood and urine shortly after exposure to 24 ppm of NO_2.[25] Increased respiration was shown in some studies. Other

Table 8.1 Health Effects Associated with NO_2 Exposure in Epidemiological Studies

Health effect	Mechanism
Increased incidence and severity of respiratory infections	Reduced efficacy of lung defenses
Reduced lung function	Airway and alveolar injuries
Respiratory symptom	Airway injury
Worsening clinical status of persons with asthma, chronic obstructive pulmonary disease or other chronic respiratory conditions	Airway injury

Source: adapted from Romieu, in *Urban Traffic Pollution*, Ecotox/WHO/E&FN Spon, London, 1999, p.9.

physiological alterations include a slowing of weight gain and decreased swimming ability in rats, alteration in blood cellular constituents, such as polycythemia, lowered hemoglobin content, thinner erythrocytes, leukocytosis (an increase in the number of leukocytes in the circulating blood), and depressed phagocytic activity. Methemoglobin formation occurred only at high concentrations. Methemoglobinemia is a disorder manifested by high concentrations of methemoglobin in the blood. Under this condition, hemoglobin contains an Fe^{3+} ion and is thus unable to combine reversibly with molecular oxygen. The lipid material extracted from the lung of rats exposed to NO_2 has revealed that oxidation had occurred. Lipid peroxidation was more severe in animals fed a diet deficient in vitamin E.[27] In contrast to O_3, reaction of NO_2 with fatty acids appears to be incomplete and phenolic antioxidants can retard the oxidation from NO_2.

Exposure to NO_2 may cause changes in the molecular structure of lung collagen. In a series of studies, Buckley and Balchum[28,29,30] showed that exposure for 10 weeks or longer at 10 ppm, or for 2 hours at 50 ppm, increased both tissue oxygen consumption and the activities of lactate dehydrogenase and aldolase. Stimulation of glycolysis has also been reported.

8.4 OZONE

8.4.1 Sources

By far the most important source of O_3 contributing to atmospheric pollution is photochemical smog. As discussed in the Section 8.3.2, disruption of the photolytic cycle of NO_2 (Reaction 8.6, Reaction 8.7, Reaction 8.8, Figure 8.4) by atmospheric hydrocarbons is the principal cause of photochemical smog.

$$NO_2 \overset{h\nu}{\rightleftharpoons} NO + O \tag{8.6}$$

$$O + O_2 \rightleftharpoons O_3 \tag{8.7}$$

$$\text{Net: } NO_2 + O_2 \rightleftharpoons NO + O_3 \tag{8.8}$$

In the above reactions, the back reaction theoretically proceeds faster than the forward reaction, and so the resulting O_3 should be removed from the atmosphere. However, free radicals formed from hydrocarbons (e.g., $RO_2^{\cdot}$, where R represents a hydrocarbon group) and other species occurring in the urban atmosphere react with and remove NO, thus preventing the back reaction. Consequently, O_3 builds up. A large number of free radicals occur in the atmosphere, such as hydroxy radical ($OH^{\cdot}$), hydroperoxy radical ($HO_2^{\cdot}$), atomic oxygen (O^1D), and higher homologs $RO^{\cdot}$ and $RO_2^{\cdot}$. Free radicals participate in chain reactions, including initiation, branching, propagation, and termination reactions in the atmosphere. The $OH^{\cdot}$–$HO_2^{\cdot}$ chain is particularly

effective in oxidizing hydrocarbons and NO. Some examples illustrating these reactions are shown below:

$$OH^{\cdot} + RH \rightarrow R^{\cdot} + H_2O \tag{8.9}$$

$$R^{\cdot} + O_2 \rightarrow RO_2^{\cdot} \tag{8.10}$$

$$RO_2^{\cdot} + NO \rightarrow RO^{\cdot} + NO_2 \tag{8.11}$$

$$RO^{\cdot} + O_2 \rightarrow R'CHO + HO_2^{\cdot} \tag{8.12}$$

$$HO_2^{\cdot} + NO \rightarrow NO_2 + OH^{\cdot} \tag{8.13}$$

It is noticeable that the process starts with an $OH^{\cdot}$ radical. After one pass through the cycle, two molecules of NO are oxidized to NO_2. The $OH^{\cdot}$ radical formed in the last step (Reaction 8.13) can start the cycle again. O_3 may also be formed from reactions between O_2 and hydrocarbon free radicals, as shown in the reaction below:

$$O_2 + RO_2^{\cdot} \rightarrow O_3 + RO^{\cdot} \tag{8.14}$$

8.4.2 Photochemical Smog

Hydrocarbon free radicals (e.g., $RO_2^{\cdot}$) can react with different chemical species, including NO, NO_2, O_2, O_3, and various hydrocarbons, such as Reaction 8.15:

$$ROO^{\cdot} + NO \rightarrow RO^{\cdot} + NO_2 \tag{8.15}$$

The hydrocarbon free radicals can also react with O_2 and NO_2 to produce peroxyacyl nitrate (PAN):

$$ROO^{\cdot} + NO_2 + O_2 \rightarrow R-\underset{\text{PAN}}{\overset{\overset{O}{\|}}{C}}-O-O-NO_2 \tag{8.16}$$

or

$$RO_3^{\cdot} + NO_2 \rightarrow RO_3NO_2 \tag{8.17}$$

It can be seen from the above discussion that a large number of chemical reactions occur in the atmosphere and result in the formation of many secondary air pollutants. In areas such as Los Angeles, where there is abundant sunshine and unique topographical conditions, these pollutants accumulate and produce smog. Air pollution problems like those found in Los Angeles and Mexico City are common among large cities of the world. The principal components of photochemical smog are O_3 (up to 90%), NO_x (mainly NO_2, about 10%), PAN (0.6%), free radical forms of oxygen, and other organic compounds, such as aldehydes, ketones, and alkyl nitrates (Table 8.2). [31]

Table 8.2 Compounds Observed in Photochemical Smog

Compound	Typical (or maximal) concentration reported (ppm)
Ozone (O_3)	0.1
PAN ($CH_3COO_2NO_2$)	0.004
Hydrogen peroxide (H_2O_2)	(0.18)
Formaldehyde (CH_2O)	0.04
Higher aldehydes (RCHO)	0.04
Acrolein (CH_2CHCHO)	0.007
Formic acid (HCOOH)	(0.05)

Source: adapted from: NAS/NRC. *Ozone and Other Photochemical Oxidants*. Committee on Medical and Biologic Effects of Environmental Pollutants. National Academy of Sciences, 1977.

8.4.3 Effects on Plants

Studies on the effects of O_3 on higher plants are extensive. Effects highlighted by the experimental results include:

- either an increase or a decrease in plant growth[32]
- decrease in size, weight, and number of fruits[33]
- decrease in shoot and root growth[34,35]
- decrease in seed oil[35]
- decrease in growth ring size[36]
- decrease in net photosynthesis[37]
- decrease in unsaturated fatty acids[38]
- increase in membrane permeability[39]
- increase in respiration[40]
- altered intermediary metabolism

The effect of O_3 on plant metabolism is complex. However, it is well established that photochemical oxidants such as O_3 and PAN can oxidize –SH groups, and such oxidation may adversely affect enzyme activity. Examples include O_3-induced inhibition of several enzymes involved in carbohydrate metabolism, such as phosphoglucomutase and glyceraldehyde-3-phosphate dehydrogenase. The hydrolysis of reserve starch in cucumber, bean, and monkey flower was inhibited by exposure to 0.05 ppm O_3 for 2 to 6 hours,[40] suggesting an inhibitory effect on amylase or phosphorylase. While decrease in glyceraldehyde-3-phosphate dehydrogenase activity suggests inhibition of glycolysis, an increase in the activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase reported by some workers implies elevated activity of the pentose phosphate pathway.[41] Recent studies indicate that exposure of mung bean seedlings to 0.25 ppm of O_3 for 2 hours markedly inhibited invertase activity.[42]

Exposure to O_3 also interferes with lipid metabolism. For instance, lipid synthesis, requiring NADPH and ATP, is known to proceed at a lower rate, presumably because O_3 lowers the total energy of the cell. O_3 also causes

ozonization of fatty acids. When O_3 reacts with a polyenoic fatty acid, for instance, the breakdown products include H_2O_2 and malonaldyde.[43] The structures of amino acids and proteins are also altered when these substances are exposed to O_3. Various amino acids, including methionine, tyrosine, cysteine, and tryptophan, are oxidized when exposed to O_3. For example, the oxidation of methionine leads to methionine sulfoxide formation in a concentration-dependent manner.[44]

8.4.4 Effects on Animals and Humans

Ozone and other photochemical oxidants cause irritation of the respiratory tract and the eye. The threshold limit value (TLV) for O_3 in industry is 0.1 ppm. Exposure to 0.6 to 0.8 ppm O_3 for 60 minutes resultes in headache, nausea, anorexia, and increased airway resistance. Coughing, chest pain, and a sensation of shortness of breath were shown in the exposed subjects who were exercised.[45] Exposure of laboratory animals to 0.7 to 0.9 ppm O_3 may predispose or aggravate a response to bacterial infection. Morphological and functional changes occur in the lung in laboratory animals subjected to prolonged O_3 exposure. Such changes as chronic bronchitis, bronchiolitis, and emphysematous and septal fibrosis in lung tissues have been observed in mice, rabbits, hamsters, and guinea pigs exposed daily to O_3 at concentrations slightly above 1 ppm. Thickening of terminal and respiratory bronchioles was the most noticeable change. For example, in the small pulmonary arteries of rabbits exposed to O_3, the walls were thicker and the lumens were narrower than those of the controls. Mean ratios of wall thickness to lumen diameter were 1:4.9 for the control, and 1:1.7 for the exposed animals.[46] This indicates that the width of the lumen of exposed animals was only about one third that of the controls.

As noted in Chapter 7, emphysema is a disease in which the alveoli in the lungs become damaged. The disorder causes shortness of breath and, in severe cases, can lead to respiratory or heart failure. Although emphysema is caused mainly by cigarette smoking, atmospheric pollution due to O_3 and some other pollutants are considered to be predisposing factors. Inhaled O_2 is passed through the thin walls of alveoli, into the bloodstream, and CO_2 is removed from the capillaries to be breathed out. Tobacco smoke and other air pollutants are believed to cause emphysema by provoking the release of chemicals within the alveoli that damage the alveolar walls. As the disease progresses, the alveoli burst and form fewer, larger sacs with less surface area, and so O_2 and CO_2 exchange is impaired (Figure 7.2b).

Other physiological effects include dryness of upper airway passages, irritation of mucous membranes of nose and throat, bronchial irritation, headache, fatigue, and alterations of visual response.

Evidence suggests that O_3 exposure accelerates the aging process. Some investigators indicate that aging is due to irreversible crosslinking between macromolecules, principally proteins and nucleic acids. Animals exposed to

0.1 ppm O_3 may increase the susceptibility to bacterial infections. Exposed mice may have congenital abnormalities and neonatal deaths.

The development of hyper-reactivity following O_3 exposure has been shown in humans and dogs. The most characteristic toxic effect of exposure to relatively high-levels of O_3 is pulmonary edema,[46] a leakage of fluid into the gas-exchange parts of the lung. This effect was seen at concentrations only slightly above that observed in pollution in Los Angeles, California.

Humans and animals have been shown to develop tolerance to O_3. *Tolerance* refers to increased capacity of an organism that has been pre-exposed to a chemical agent, such as an oxidant, to resist the effects of later exposures to ordinarily lethal, or otherwise injurious, doses of the same agent. For example, rodents exposed to 0.3 ppm O_3 would become tolerant to subsequent exposures of several ppm O_3, a dose that would produce massive pulmonary edema in animals exposed for the first time. Some human subjects exposed to 0.3 ppm O_3 at intervals of approximately one day showed diminished reactivity after later exposures. This response is termed *adaptation*.[47]

8.4.5 Biological Effects

A large volume of literature has been published describing the biochemical effects of O_3. Examples of the reported effects include:

- reactions with proteins and amino acids
- reactions with lipids
- formation of free radicals
- oxidation of sulfhydryl compounds and pyridine nucleotides
- production of more or less nonspecific stress, with the release of histamine

As mentioned in the previous section, O_3 interacts with proteins and some amino acids, altering their characteristics. In humans, the amount of lysozyme in tears of individuals exposed to smog was shown to be 60% less than normal. The concentrations of protein and nonprotein sulfhydryls in the lungs of rats exposed to 2 ppm O_3 for 4 to 8 hours were shown to be decreased. A number of investigators have shown that O_3 can cause the oxidation of the –SH group, and that addition of SH compounds was protective.

The activities of several enzymes are either enhanced or depressed in animals exposed to O_3. Reports on decreases in enzyme activities include glucose-6-phosphate dehydrogenase, glutathione reductase, and succinate-cytochrome c reductase in the lungs of rats exposed to 2 ppm O_3 for 4 to 8 hours, whereas increased activities were shown with glucose-6-phosphate dehyrogenase, 6-phosphogluconate dehydrogenase, and isocitrate dehydrogenase.

Balchum et al.[48] have provided evidence to support the concept that the peroxidation or ozonization of unsaturated fatty acids in biological membranes is a primary mechanism of the deleterious effects of O_3. The hypothesis was

based on the tendency of O_3 to react with the ethylene groups of unsaturated fatty acids, resulting in the formation of free radicals. In the presence of molecular oxygen, the free radicals can cause peroxidation of unsaturated fatty acids. It has been observed that lipid material subjected to O_3 exposure showed a relative decrease in unsaturated fatty acids as compared with saturated fatty acids, and the more unsaturated the fatty acids were, the greater the decrease observed. Furthermore, in the rat a deficiency of vitamin E increases the toxicity of O_3.[49] Possible mechanisms for O_3 toxicity involving peroxidation of membrane unsaturated fatty acids include: the ability of O_3 to react with polyunsaturated fatty acids (PUFA), causing lipid breakdown (breakdown products can include H_2O_2, carbonyl compounds, and various free radicals, which are detrimental to cells), and the resultant free radicals may react with:

- protein –SH groups, leading to enzyme inactivation
- mitochondrial PUFA, resulting in swelling and impaired energy metabolism or loss of energy metabolism
- lysosomal PUFA, causing release of lysosomal hydrolases
- nuclear PUFA, leading to carcinogenesis[50]

Another chemical pathway that can induce O_3-dependent oxidation of unsaturated fatty acids is through incorporation of O_3 into the fatty acid double bond, resulting in ozonide formation. This process is generally known as ozonolysis (Figure 8.6). Ozone is also known to oxidize GSH and pyridine nucleotides NADH and NADPH. The ozonization of the nicotinamide ring of NADPH may proceed in such a way as that shown in Figure 8.7.

Because the intracellular ratios of $NADH/NAD^+$, $NADPH/NADP^+$, and ATP/adenylates are carefully regulated by the cell, loss of the reduced nucleotide can be compensated for by faster operation of the Krebs cycle.

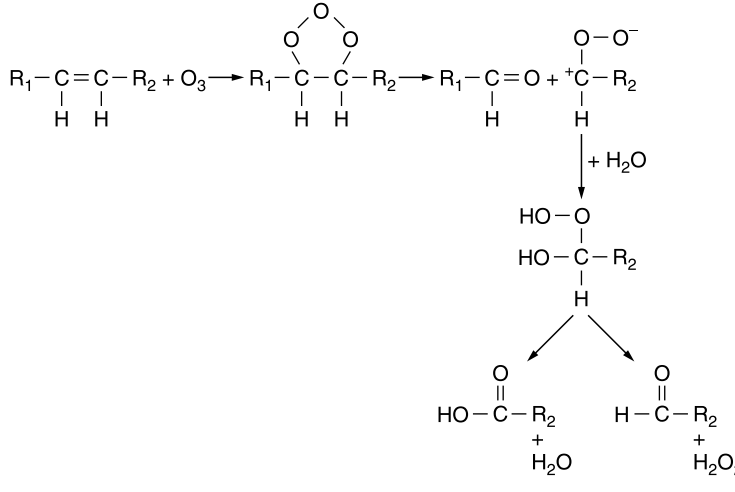

Figure 8.6 Ozonization of membrane lipids.

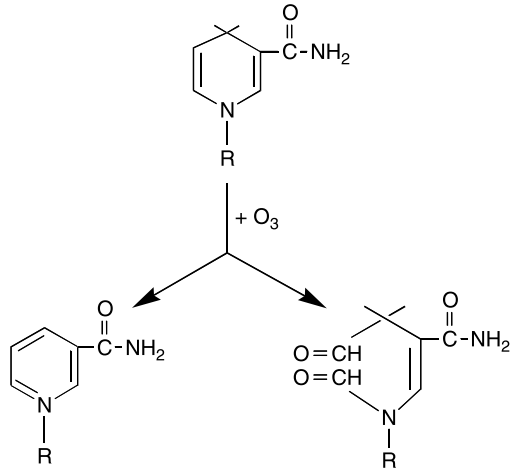

Figure 8.7 Ozonization of the nicotinamide ring in NADPH.

However, the cell can only make up for a net loss of all nucleotides by an increase in synthesis. The oxidation of NADH or NADPH results in elevated enzyme activity, which permits the cell to restore the initial ratio of the nucleotides. With NADPH, oxidation increases the activity of the pentose phosphate pathway. Such increase also occurs following the oxidation of GSH (Reaction 8.18). Oxidation of either NADPH or GSH, therefore, may be responsible for the apparent increase in enzymes in the pentose phosphate pathway after repeated O_3 exposure.

$$2\,GSH + [O] \xrightarrow{\text{GSH peroxidase}} GSSG + H_2O \qquad (8.18)$$

$$2SSG + 2NADPH \xrightarrow{\text{GSH reductase}} 2GSH + 2NADP^+ \qquad (8.19)$$

$$G\text{-}6\text{-}P + NADP^+ \xrightarrow{\text{G-6-P dehydrogenase}} 6\text{-}PG + NADPH \qquad (8.20)$$

8.5 CARBON MONOXIDE

8.5.1 Introduction

Carbon monoxide (CO) is an odorless, colorless, and tasteless gas found in high concentrations in the urban atmosphere. No other gaseous air pollutants with such a toxic potential exist at such high concentrations in urban

environments. Historically, early exposures resulted from the use of wood-burning fires and then from using coal for domestic heating. Combustion of fossil fuel associated with developing industry, explosions, fires in mines, and illumination gas prepared from coal all have been sources of exposure. The migration of agricultural populations to cities increased the proportion of exposed population, as well as the number of persons generating CO. With the emergence of automobiles propelled by internal combustion engines, CO emitted from exhaust pipes has become the major source for human exposure. Serious problems also exist due to occupational exposure to increased levels of CO.

8.5.2 Formation

Carbon monoxide is usually formed through one of the following three processes: incomplete combustion of carbon-containing fuels, reactions between CO_2 and carbon-containing materials at high temperature, and dissociation of CO_2 at high temperatures.

Incomplete combustion of carbon or carbon-containing compounds occurs when the available oxygen is less than the amount required for complete combustion, in which CO_2 would be the product (Reaction 8.21 and Reaction 8.22). It will also occur when there is poor mixing of fuel and air.

$$2C + O_2 \rightarrow 2CO \tag{8.21}$$

$$2CO + O_2 \rightarrow 2CO_2 \tag{8.22}$$

Carbon monoxide is also produced when CO_2 reacts with carbon-containing materials at an elevated temperature (Reaction 8.23). Such reactions are common in many industrial devices.

$$CO_2 + C \rightarrow 2CO \tag{8.23}$$

The CO produced in this way is utilized in a variety of industrial facilities, such as the blast furnace of a smelter, where the CO acts as a reducing agent in the production of iron from Fe_2O_3 ores (Reaction 8.24). Some CO may, however, escape into the atmosphere.

$$3CO + Fe_2O_3 \rightarrow 2Fe + 3CO_2 \tag{8.24}$$

CO may also be produced by the dissociation of carbon dioxide into CO and O at high temperatures, as shown in Reaction 8.25.

$$CO_2 \xrightarrow{\text{High temperature}} CO + O \tag{8.25}$$

8.5.3 Human Exposure

Human exposure to CO occurs mainly from three sources: ambient air, occupational exposure, and cigarette smoke.

CO in the surrounding ambient environment is largely emitted in exhaust gases (automobiles, industrial machinery), but other sources of accidental intoxication include house fires (which may contain more than 50,000 ppm CO) and environmental problems in the house (such as defective furnaces, charcoal burning in poorly vented houses, or garages connected to living quarters).

Individuals particularly at risk from occupational exposure include fire fighters (> 10,000 ppm CO), traffic police, coal miners, coke-oven and smelter workers, tollbooth attendants, and transportation mechanics.

8.5.4 Health Effects

A constant supply of O_2 is needed in order for physiological functions to proceed normally in the body. Oxygen is carried to body tissue by hemoglobin (Hb), a complex component of red blood cells that consists of two pairs of proteins (α and β chains), which themselves are bonded around an iron. Hemoglobin picks up O_2 in the lungs, forming a complex called oxyhemoglobin (HbO_2), as shown below:

$$Hb + O_2 \rightarrow HbO_2 \tag{8.26}$$

Once the HbO_2 reaches the body tissues, it releases the bound O_2 to be used:

$$HbO_2 \rightarrow Hb + O_2 \tag{8.27}$$

The Hb is then returned to the lungs for a new supply of O_2.

CO is toxic because it enters the bloodstream and reduces the ability of the red blood cells to deliver oxygen to the body's organs and tissues. The toxic action of CO involves the formation of carboxyhemoglobin (COHb or HbCO):

$$CO + Hb \rightleftarrows HbCO \tag{8.28}$$

The chemical affinity of CO for Hb is more than 200 times greater than that of O_2. Furthermore, in the presence of CO, HbO_2 readily releases the bound O_2 and picks up CO to form HbCO:

$$HbO_2 + CO \rightleftarrows HbCO + O_2 \tag{8.29}$$

Because the binding sites of each polypeptide chain on the hemoglobin molecule cannot be occupied by the O_2 and CO at the same time, it is apparent

that CO can tie up a substantial quantity of Hb when HbCO is formed. Consequently, Hb will not be able to transport O_2 to tissues, thus severely impairing bodily function, especially of the heart and central nervous system.

Although increase in oxygen concentrations can shift the equilibrium in Reaction 8.29 to the left, recovery of Hb is slow, while the asphyxiating effect of binding Hb with CO is rapid. People with cardiovascular disease, particularly those with angina or peripheral vascular disease, are much more susceptible to the health effects of CO. Furthermore, research showed that the fetus is particularly susceptible to lack of O_2 supply, therefore maternal CO poisoning during pregnancy can lead to fetal death. Animal studies have shown that the offspring of pregnant female rats exposed to CO have lower birth weights and significant learning deficits.[51]

The normal or background level of blood HbCO is about 0.5%. Part of the CO in background HbCO is derived from the ambient air, while the rest is originated by the body as a result of heme catabolism. The equilibrium percentage of HbCO in the bloodstream of a person continually exposed to an ambient air CO concentration of less than 100 ppm can be calculated from the following equation:

$$\text{HbCO in blood (\%)} = 0.16 \times (\text{CO conc. in the air in ppm}) + 0.5$$

According to available data (Table 8.3),[27] the concentration of HbCO in the blood required to induce a decreased O_2 uptake capacity is approximately 5%. Impairment in the ability to correctly judge slight differences in successive short time intervals has been observed at HbCO levels of 3.2 to 4.2%. The most well-known symptoms of CO poisoning are headache and dizziness, which occur at HbCO levels between 10 and 30%. At levels above 30%, the symptoms are severe headache, cardiovascular symptoms, and malaise. Above about 40%, there is considerable risk of coma and death.[27] In case of acute CO poisoning, 100% oxygen is commonly used to treat the victim.

Table 8.3 Human Health Effects Associated with Carboxyhemoglobin (HbCO) Levels

HbCO level (%)	Health effects
<1.0	No apparent effect
2–4	Impairment of visual function; decreases in the relation between work time and exhaustion in exercising young healthy adults
2.0–4.5	Decrease in exercise capacity in patients with angina
<5	Vigilance decrement
5–5.5	Decrease in maximum oxygen consumption and exercise in young healthy men during strenuous exercise
5–17	Impairment of visual perception, of manual dexterity, of learning ability or performance of certain intellectual tasks
20–25	Nausea, weakness (particularly in the legs), occasional vomiting

Source: Pereira, L.A. et al., *Environ. Health Perspect.*, 106, 325, 1998.

The half-life of HbCO is estimated to be 4 hours at rest at room air, and it is shortened to 60 to 90 minutes if 100% oxygen is given using a facemask. In addition to its association with Hb in red blood cells, CO binds to other proteins in the body, such as myoglobin, cytochrome c oxidase, and cytochrome P450, thereby impairing their action. CO also inhibits alveolar macrophage function, weakening tissue defenses against airborne bacterial infection.

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8.7 REVIEW QUESTIONS

1. Briefly explain the role that the free hydroxyl radical ($OH^{\cdot}$) plays in the atmosphere.
2. Explain the chemical changes that occur once SO_2 is absorbed into a plant leaf.
3. Compare the phytotoxicity of S-containing chemical species produced from SO_2 in a plant leaf.
4. What could be the basis for different plant species to exhibit different sensitivity to SO_2?
5. How is SO_2 exposure related to respiratory system in animals and humans?
6. Explain the toxic effect of acidic aerosol inhalation in humans.
7. Describe the photolytic cycle of NO_2.
8. Explain the way in which plants may make use of dissolved NO_x.
9. What can you tell from the observation that lipid peroxidation is more severe in animals whose dietary vitamin E is deficient?

10. Describe the mechanism whereby ozone may accumulate in the troposphere.
11. What is photochemical smog? What are the main components?
12. Explain how O_3 may injure membrane structure.
13. What is PAN? Explain how it may be formed.
14. What effect does O_3 have on amino acids?
15. What is the characteristic respiratory problem that an emphysematous patient may suffer?
16. What is the most noticeable change in the bronchioles of animals exposed to O_3?
17. What is pulmonary edema? Which air pollutant(s) can cause it?: SO_2, NO_2, F, O_3.
18. Draw a diagram to illustrate the biochemical effect of O_3 on membrane unsaturated fatty acids.
19. What is ozonolysis? Briefly explain the process involved.
20. Write a chemical equation to show the reaction between O_3 and GSH.
21. Explain how CO may be formed from CO_2.
22. What is carboxyhemoglobin? Write down a chemical equation to show its formation.
23. What is the physiological basis for the toxicity of CO?
24. Explain how CO may be related to macrophage function.
25. What is the relationship between CO and cytochrome P450?

Chapter 9

Air Pollution – Particulate Matter

9.1 INTRODUCTION

Particulate air pollution refers to the presence of small solid and liquid aerosols suspended in the atmosphere. The physical dimensions and chemical properties of these aerosols vary greatly. Their sizes may vary from 0.5 mm to 10^{-7} mm, and they are composed of a large number of inorganic and organic materials, including metals and nonmetal elements (and their oxides, nitrates, and sulfates). Although it is often convenient to group them as *particulates*, their sources, distribution, and effects can be highly variable. Because of the large quantities of particulates emitted into the atmosphere and the potential adverse effects they elicit, the U.S. Environmental Protection Agency (EPA) has designated particulate matter (PM) as one of the six "Criteria Air Pollutants" to be regulated. In 1987, the agency added a new standard for particulates, called PM_{10} (referring to PM with a diameter less than 10 μm), based on the evidence that the smaller PM has the greatest impact on health because of its capacity to be inhaled. This chapter presents an overview of this class of air pollutants, followed by discussion of three specific examples of PM: silica, beryllium, and asbestos.

9.2 CHARACTERISTICS

Particulates are usually classified into primary or secondary. Primary particulates are larger (usually 1 to 20 μm in diameter) and are emitted directly into the atmosphere by a variety of chemical and physical processes. Secondary particulates are relatively smaller and are formed by chemical reactions occurring in the atmosphere.[1] The composition of particulates varies from place to place, and includes thousands of entities that differ in size, surfaces, and toxicity.[2] Particles in most urban aerosols have been shown to contain a number of potentially toxic trace species, such as lead (Pb), cadmium (Cd), nickel (Ni), selenium (Se), vanadium (V), zinc (Zn), bromine (Br), cobalt (Co), manganese (Mn), sulfate, and benzo[a]pyrene.[3]

Evidence from recent studies strongly suggests the importance of the primary urban aerosols. It is considered that even though these primary aerosols contribute a minor amount to the total mass of aerosols, they serve as condensation nuclei upon which the secondary aerosol mass resides and they carry the bulk of the particulate toxins.[4]

9.3 FORMATION OF PARTICULATES

Particulates are formed from both natural and anthropogenic sources. Natural sources include volcanic ash, wildfire particles, fine soil particles, fine marine salts from ocean spray, biological particles such as pollen, fungal spores, etc. Fine particulates are also produced by atmospheric reactions among gases, such as through photochemical reactions. Anthropogenic sources include a variety of industrial combustion processes, mining, vehicle emissions, domestic heating and cooking, pottery making, metalworking, and many other manufacturing processes. In addition, cultivation of agricultural land also contributes significant amounts of PM, through land clearance and fire control activities.

9.3.1 Physical Processes

Particulate matter can be formed through both physical and chemical processes. Particles above approximately 1 mm in size are generally formed by the disintegration of larger particles. This is called a dispersion process, and the product is known as a dispersion aerosol. Dusts are solid dispersion aerosols, which may be formed through a variety of natural and human activities. Some examples include: volcanic eruption, wind-blown dust from dry soil, ocean spray, coal grinding, rock crushing, stone cutting and polishing, high-power drilling of tunnel rocks, and manufacture of pottery.

9.3.2 Chemical Processes

Both inorganic and organic particles are produced through various chemical processes. Metal oxides form a major class of inorganic particles in the atmosphere. They are produced whenever fuels containing metals are burned. For instance, particulate iron oxide is produced in the combustion of coal that contains iron sulfide (FeS_2) as a contaminant:

$$3FeS_2 + 8O_2 \rightarrow Fe_3O_4 + 6SO_2 \tag{9.1}$$

As noted in Section 8.2, sulfuric acid (H_2SO_4) mists are formed from the oxidation of atmospheric sulfur dioxide (SO_2):

$$2SO_2 + O_2 + 2H_2O \rightarrow 2H_2SO_4(\text{liquid droplets}) \tag{9.2}$$

The sulfuric acid thus formed can react with basic air pollutants, such as ammonia (NH_3), calcium oxide (CaO), lead oxide (PbO), or aluminum oxide (Al_2O_3), forming various sulfates:

$$H_2SO_4(\text{droplet}) + 2NH_3(\text{gas}) \rightarrow (NH_4)_2SO_4(\text{droplet}) \tag{9.3}$$

$$H_2SO_4(\text{droplet}) + PbO(\text{particle}) \rightarrow PbSO_4(\text{droplet}) + H_2O \tag{9.4}$$

Combustion of leaded gasoline results in the formation of, among other substances, several kinds of lead halides. Tetraethyl lead ($Pb(C_2H_5)_4$ in leaded gasoline reacts with molecular oxygen (O_2) and halogenated scavengers, such as dichloroethane and dibromoethane, producing various forms of lead halide particles that are emitted into the atmosphere:

$$Pb(C_2H_5)_4 + O_2 + (\text{halogenated scavengers}) \rightarrow CO_2 + H_2O + PbCl_2 + PbBrCl + PbBr_2 \quad (9.5)$$

Additionally, whole gasoline vapor alone has been shown to contribute significantly to atmospheric aerosol formation.[5]

9.4 HEALTH EFFECTS

Fly-ash particles are generally composed of stable elements or compounds that are usually not considered directly toxic in concentrations found in ambient air. However, subtle toxicity has been recognized under some conditions. In particular, many trace elements have important biological activity and are, therefore, potential health hazards.

The toxicity of PM generally arises from any of the following factors. The particles may themselves be toxic, e.g., particles containing toxic metals and nonmetals, such as Pb, Cd, Ni, mercury (Hg), arsenic (As), and radionuclides[6, 7] Alternatively, the particles may adsorb toxic chemicals, such as carcinogens,[7,8] and enhance their effect by either increasing their penetration into the lungs, or prolonging their residence time in the respiratory tract. Particles may also serve as condensation nuclei for water and other vapors, producing droplets and enhancing biological effects. Finally, if there are large quantities present in the respired air, particles may overtax the mucociliary apparatus, thus decreasing the rate of removal of toxic chemicals from the lung.

Many occupational activities cause the formation of dust. Mining, metal grinding, and sand blasting have been shown to cause *pneumonoconiosis*, a disease of the lung caused by habitual inhalation of irritant mineral or metallic particles. The disease is characterized by fibrous degeneration known as *fibrosis*. Several factors contribute to the development of pneumonoconiosis, and those related to workers and to dust are particularly important. The factors pertaining to workers include duration of exposure to dust and the susceptibility of individual workers. The size of particles, their chemical composition, and their concentrations are also important. Researchers have identified various specific diseases, based on the chemical elements involved in fibrosis formation. For example, *silicosis* results from inhalation of silicon oxide (SiO_2); *silicatosis,* from silicate; *siderosis*, from hematite (Fe_2O_3); *talcosis*, from talc ($Mg_3Si_4O_{10}(OH)_2$); and *bariosis*, from barium (Ba).

The size of particles is very important in the pathogenesis of pneumonoconiosis. This is because the size affects the concentration of particles that may

be suspended in the air, and may determine the depth to which the particles penetrate into the lung, and so the amounts that may be deposited and retained.

In addition to widely recognized occupational health effects of PM, numerous epidemiological studies have confirmed that total suspended particles (TSP) present in urban areas, especially those < 2.5 μm in diameter, were associated with increased risk of mortality in pneumonia and cardiovascular disease. The risk is particularly elevated in the elderly.[6]

The limited information available on the rainforest fire episode in Indonesia in the summer of 1997 indicates widespread acute impacts on the health of a large number of the population. According to the Singapore Ministry of Health, there was a 13% increase in visits to government clinics for acute respiratory infections and a 19% increase in asthma visits during the last week of September when PM levels peaked.[9]

China has achieved high rates of economic growth during the past two decades or so. The growth is associated with rapid industrialization, accelerated urbanization, and greatly increased energy consumption.[10] The accelerated urbanization is evidenced by the steady increase in the proportion of urban population to total population, from 18% in 1978 to 31% in 1999, a rate three times the world average during this period. The explosive economic growth also made China the world's second largest energy consumer, after the U.S. Between 1978 and 1999, China's energy consumption more than doubled, and is the main source of anthropogenic air pollution emissions in Chinese cities. Coal accounted for about 74% of the total energy consumption during this period. The increased use of coal is considered the origin of many air pollution problems, including SO_2 pollution, PM, and acid rain.[10]

Studies by Xu et al.[11] show that the air-pollution levels in Beijing are associated with adverse health outcomes. The scientists studied the data on the average number of daily hospital outpatient visits at a community-based hospital in Beijing, and compared the data with the levels of SO_2 and TSP in the atmosphere. They found that increases in the levels of the two types of pollutants were significantly correlated with the increases in the numbers of hospital visits.

A similar observation was made in Seoul, South Korea, where several scientists investigated the impact of air pollution on human health. For example, Ha et al.[12] studied the effect of air pollution on mortality among postneonates, those aged 2 to 64 years, and those over 65 years of age. The study included daily counts of total deaths and respiratory-related deaths, along with analyses of daily levels of PM_{10}. The results showed that in terms of mortality, infants were most susceptible to PM_{10}, particularly where deaths were related to the respiratory system.[12]

9.5 SILICA

Silica (Silicon dioxide, SiO_2) and silicates constitute the major portion of all rocks and their products, such as soils, sands, and clays. Silicon (Si) itself is the

second most abundant element (after oxygen) in the earth's crust. SiO_2 occurs in either its free form or a combined state called *silicate*. Free silica may be in crystalline form, such as quartz, granite, flint, and diatomite, or in noncrystalline form.

9.5.1 Silicosis

Silicosis is a disease caused by breathing tiny particles of crystalline free SiO_2. It is considered the most important of the pneumonoconiosies, or dust diseases of the lung, not only because of its serious damaging effect on the respiratory system but also because of the large numbers of workers throughout the world who are at risk of contracting it. Acute silicosis is manifested within 8 to 18 months following the first exposure. Chronic silicosis may develop after a latent period of up to 20 years. It is found among people with occupational exposure to breathable crystalline free silica, for example in mining industries, pottery manufacture, stone cutting and polishing, tile and clay production, and glass manufacture. Silicosis increases susceptibility to various respiratory infections, notably tuberculosis.

The size of the silica or silicate particles has a big effect on the degree of tissue reaction that will occur following the inhalation of the siliceous dust. Particles of 0.5 to 10 μm diameter are responsible for the disease because they lead to fibrogenic reaction in alveolar tissue. Fibrous, or scar, tissue is formed, replacing the normal lung tissue. However, the fibrous tissue does not have the elasticity of normal tissue and so limits the lung's ventilatory function and the exchange of gases between the air and blood. As a result, the victim becomes short of breath, a principal clinical characteristic of silicosis.

9.5.2 Pathogenesis

Many hypotheses have been advanced to explain the mode of action of silica pertaining to its fibrogenic properties. For many years, researchers thought the fibrogenic properties were due to the action of silicic aid (H_4SiO_4). However, Allison et al.[13] suggested that the intracellular reaction to silicic acid is the first stage of a two-stage process in which the major fibrogenic stimulus comes from the action of cellular enzymes rather than directly from the silicic acid itself. According to their hypothesis, once a silica particle is arrested in the lungs, it is invaginated leading to phagocytosis. The particle is encapsulated within the cell in a phagosome, which soon becomes converted into a second type of lysosome (digestive vacuole) through merging with a primary lysosome, presumably secreted by the Golgi body. Protective substances adsorbed onto the silica particles (e.g., plasma proteins) are stripped off by the enzymes released from the lysosome, exposing silicic acid. The highly reactive silicic acid acts as a hydrogen donor, forming hydrogen-bonded complexes with active groups of the lipid membrane, such as phosphate ester groups, and with secondary amide (peptide) groups of proteins. This reaction causes the lysosomal membrane to become permeable, allowing its enzymes to leak into the cytoplasm and destroy

the cell. With cellular dissolution, the cell contents, including active lysosomal enzymes, along with the ingested silica, are released into the tissue interstices (Figure 9.1). Moreover, the freed particles of silica are again phagocytosed by other macrophages, resulting in a chain of events.

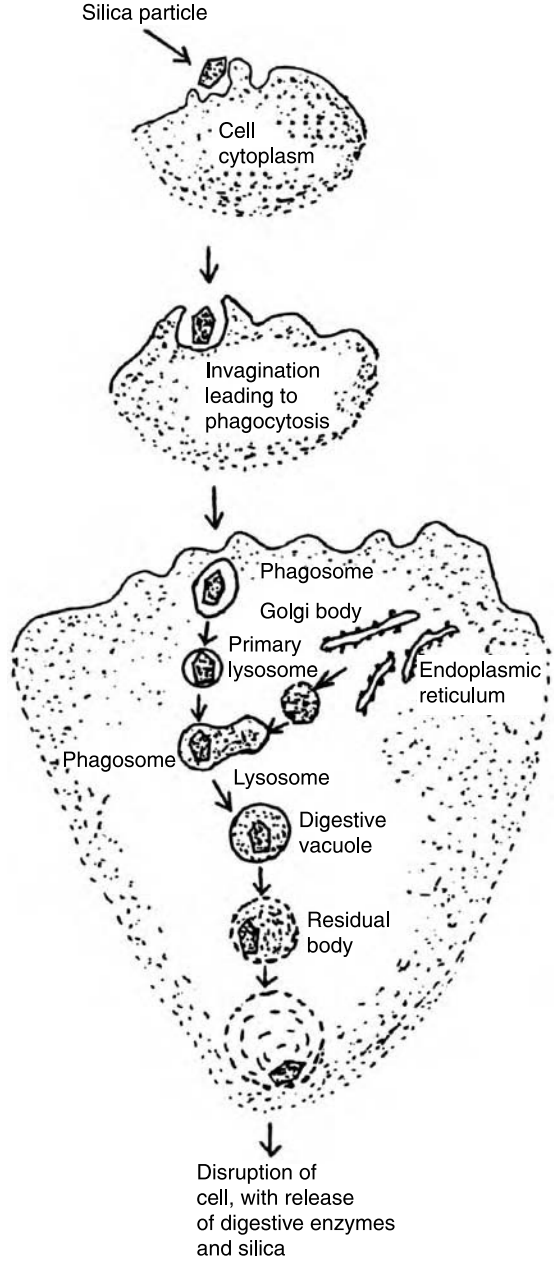

Figure 9.1 Suggested mechanism of silicosis.
Source: adapted from Allison, A.C. et al., *J. Exp. Med.*, 124, 141, 1966.

9.6 BERYLLIUM

Beryllium (Be) is one of the least-known environmental pollutants, and yet it is one of the most toxic nonradioactive elements known. Its industrial threshold limit value (TLV) is 2 mg/m^3, the lowest for any particulates. (The TLV of a toxicant is defined as the maximum concentration to which it is believed healthy workers may be repeatedly exposed without ill effect, based on an 8-hour working day.) The toxicity of Be in humans was described in the U.S. in the 1940s, when more than 500 cases of Be disease were reported. Acute disease occurred in Ohio among Be extraction and production workers, while chronic disease was found in Massachusetts among workers manufacturing fluorescent lamps containing beryllium-phosphor. The use of Be in fluorescent lamps was discontinued in 1950. As a result, Be disease incidence has decreased dramatically.

Be is estimated to make up about 0.0006% of earth's igneous rocks. Of 28 minerals in which Be is a minor accessory constituent, *beryl* (beryllium aluminum silicate, $Be_3Al_2Si_6O_{18}$), with 14% BeO, is the chief source of Be, and as such is the most important commercially. Be is also found in coal, in amounts ranging from 0.1 to 1000 ppm.

9.6.1 Sources of Exposure

Exposure to Be compounds can occur within the production and manufacturing industries, as well as in housekeeping, maintenance, salvage, and solid-waste areas. Individuals working in any operations involving production of airborne Be are at risk for developing Be disease. The major occupations at risk of developing Be disease have changed since the first reports of cases in the U.S. in the 1940s. Before 1950, exposure most commonly occurred in fluorescent lamp manufacturing, atomic bomb research, and Be extraction operations. After 1950, Be was replaced in fluorescent lamp production by a calcium phosphor. However, Be has since been used in modern technologies, including nuclear reactors, electronics equipment, guidance and navigation systems, rocket parts, and heat shields. It is employed extensively as an alloying agent for copper as it adds tensile strength, conductivity, and corrosion resistance.

Chronic Be disease has been reported in people living in areas adjacent to a plant or industry using the metal, suggesting neighborhood exposure from plant discharges into the air. It has also been shown that families of Be workers may also be exposed to the metal as a result of dust carried home on the workers' clothes. Between 1973 and 1980, 66 cases of chronic Be disease were reported in the U.S., nearly half of these involved Be metal production. The combustion of coal is considered the largest source of environmental Be contamination. Some coal contains about 2.5 ppm, and oil contains about 0.8 ppm of Be. Atmospheric emission of Be from these sources has been estimated to be above 1000 t annually.[14]

9.6.2 Health Effects

Chronic Be disease, commonly known as berylliosis, is manifested by pulmonary and systemic granulomatous disease caused by exposure to Be by inhalation. The duration of exposure may be from several months to years. The interval between initial exposure and clinical manifestations of disease varies with individuals. Some patients may not become symptomatic until up to 25 years after their last exposure. The average latency is 10 to 15 years. The most common symptom of chronic Be disease is dyspnea (shortness of breath). Other symptoms include cough, fatigue, weight loss, chest pain, signs of pulmonary hypertension, nodular skin lesions, and conjunctivitis.

In acute Be disease, nasopharyngitis, tracheobronchitis, or chemical pneumonitis may occur, resulting in edema, inflammation, and necrosis. The severity of clinical disease depends largely on the dose of Be exposure. Symptoms and signs are nonspecific, identical to those found in any case of chemical pneumonitis secondary to a lung irritant, and include dyspnea, cough, chest pain, blood-tinged sputum, and cyanosis. Acute Be disease is currently uncommon. Evidence from both animal experiments and human epidemiologic studies suggests a link between Be and lung cancer in humans.

9.6.3 Biological Effects

In animal studies, Be has been shown to cause ultrastructural changes in the liver. Alterations included vacuolization and dense deposits in lysosomes, loss of fibrils and appearance of dense plaques in some nucleoli, and distortion of bile canaliculi.[15] Changes in lysosomal morphology were found to correlate with the biochemical evidence of localization of Be within lysosomes. Increases in serum gammaglobulins, elevated erythrocyte sedimentation rate, and erythrocytosis, hyperuricemia, and transient hypercalcemia and hypercalciuria, have also been noted.

Be affects the enzyme that leads to DNA synthesis and can act as a competitive inhibitor of magnesium (Mg^{2+}), a cofactor for DNA polymerase. DNA polymerase catalyzes the formation of a polynucleotide from a single DNA template strand and a short complementary DNA or RNA primer. It also functions to "proofread" the base pairing as a new strand of DNA is formed. In this way, it can remove an incorrectly base-paired nucleotide before the next nucleotide is added to the DNA strand. Therefore, when Be competitively inhibits Mg^{2+}, a base-substitution mutation may occur. It has been reported that the physical properties of DNA are affected by 0.1 to 1 m*M* beryllium sulfate ($BeSO_4$).

9.6.4 Therapy

One of the remedies for berylliosis is the use of chelating agents, such as aurintricarboxylic acid (ATA) (Figure 9.2a). In an animal experiment, researchers injected mice with enough Be salt to kill them within a few days.

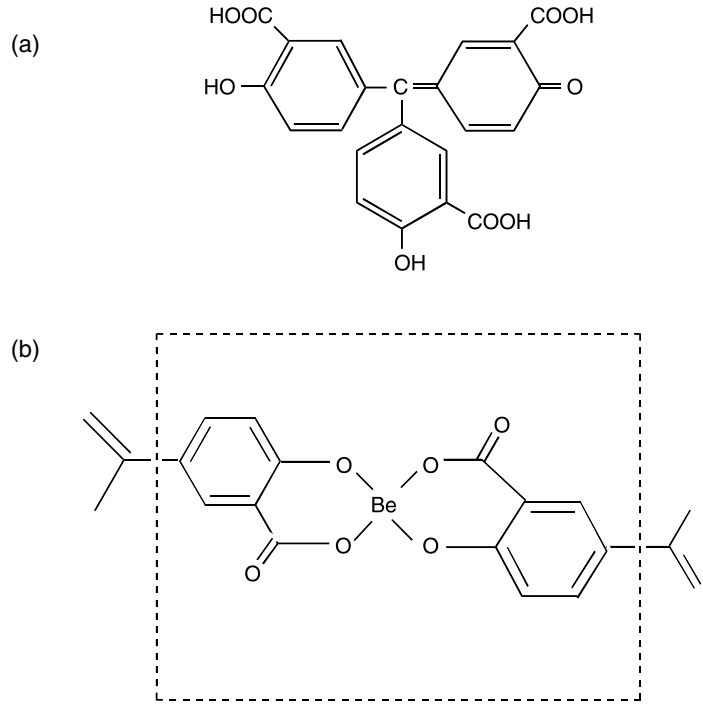

Figure 9.2 (a) Chemical structure of aurintricarboxylic acid (ATA), and (b) suggested mechanism whereby Be is chelated by ATA

Half of the animals were injected with a small quantity of ATA and the other half were left untreated. The results showed that virtually every animal treated with ATA survived and lived on normally, whereas all the untreated animals died. The experiments were repeated with hundreds of animals of different species, with the same high degree of protection. Subsequent studies using radioactive Be and ATA showed that the chelating agent was found in practically every cell where Be was present. Previously-damaged cells recovered, and within a few days they could not be distinguished from the normal tissue cells. How ATA functions chemically to antagonize Be is incompletely understood. A suggested mechanism involved in the chelate is shown in Figure 9.2b.

9.7 ASBESTOS

Asbestos is the generic name given to a specific group of six naturally occurring fibrous silicate minerals. These include the fibrous serpentine mineral chrysotile and five fibrous amphibole minerals (actinolite, amosite, anthophyllite, crocidolite, and tremolite).[16] When crushed or processed, these minerals separate into flexible fibers made up of fibrils.[17] Asbestos is mined primarily

from open pits. The annual consumption of asbestos in the U.S. peaked at about 800,000 t in 1973, but by 1984 it had declined to slightly above 200,000 t, mainly because of concerns about its toxicity.[18]

9.7.1 Chemical and Physical Properties

Asbestos minerals have a number of desirable properties that made it useful in commercial applications. These include tensile strength, heat stability, thermal and electrical insulation, wear and friction characteristics, the ability to be woven, and resistance to chemical and biological degradation.[16]

Chrysotile, the most abundant form of asbestos, occurs naturally in lengths from 1 to 20 mm. Its chemical composition is $Mg_3(Si_2O_5)(OH)_4$ and it exists as a curled sheet that forms a spiral around a central hollow tube silicate. Small amounts of Fe, Al, Ni, calcium (Ca), chromium (Cr), Mn, sodium (Na), and potassium (K) may be present as imprities. Chrysotile may be white, gray, green, or yellowish, and has a silky luster.[16]

9.7.2 Use

Although asbestos use dates back at least 2000 years, modern industrial use began around 1880. Asbestos demand peaked in the late 1960s and early 1970s, when more than 3000 industrial applications or products were listed. It has been estimated that in the U.S. more than 320,000 km of asbestos-cement pipes, carrying drinking water and other materials, had been laid. Asbestos has been used in brake linings, roofing, clutch facings, thermal and electrical insulation, cement pipes and sheets, filters, gaskets, friction materials, textiles, steam and fire hoses, plastics, gas-mask filters, paper, and other products.[16]

Consumption of asbestos in the U.S. has been declining for two decades. Reported consumption in 1980 was 360 million kg. By 1998 and 1999, U.S. consumption had declined to about 16 million kg/year. Only chrysotile is presently used for manufacturing in the U.S., most of it is used in plastics. [16]

9.7.3 Exposure

Asbestos is released to the environment from both natural and anthropogenic sources, and has been detected in indoor and outdoor air, soil, drinking water, food, and medicines. Asbestos has also been detected within the Greenland ice sheet. The primary routes of potential human exposure to asbestos are inhalation and ingestion. Worker exposure is a concern in the mining and milling of asbestos, during manufacture of all asbestos products, and in the construction and shipbuilding industries. It has been reported that in the U.S. an estimated 37,000 persons were employed in manufacture of primary asbestos products, while 300,000 persons were in secondary asbestos industries. In addition, workers may be exposed to asbestos in consumer industries, such as brake repair, asbestos insulation, and asbestos abatement. According to a 1990 Occupational Safety and Health Administration (OSHA) report,

approximately 568,000 production and service industry workers and 114,000 construction industry workers were potentially exposed to asbestos.[16]

Asbestos bodies were found in 48.3% of lungs of 3000 consecutive autopsies from three hospitals in New York. Similar observations were made in other big cities. In addition to the lungs, other organs, such as the thyroid, spleen, pancreas, heart, adrenals, kidney, prostate, brain, and liver, have also been shown to contain some asbestos.

9.7.4 Health Effects

Health problems related to exposure to asbestos were first observed by the early 1900s. It was recognized in the 1950s and 1960s that asbestos was responsible for lung and pleural tumors in asbestos miners.[19] The effects of asbestos fibers in biological systems may result not only from the properties of the fibers themselves, but also from contamination with inorganic or organic substances that occur naturally or are added during mining, milling, processing, or shipping.

The proven or suspected effects of asbestos minerals on human health include nonmalignant changes, such as pulmonary and pleural fibrosis, and several types of malignancy, especially of the lung, pleura, and peritoneum. Association between asbestos and human disease was revealed by studies on certain occupational groups, notably workers engaged in the mining and milling of asbestos, the manufacture of asbestos-containing products, and the application and removal of asbestos-containing insulating materials.

Asbestosis (or asbestotic pneumoconiosis) may develop after years of intense exposure and was the first clearly demonstrated adverse effect of asbestos in humans. The condition is characterized by pulmonary fibrosis (scarring of the lungs). Victims become increasingly short of breath, and in extreme cases may die of heart failure. Some researchers suggested that from between 1950 and 1975, approximately 10% of the deaths among New York City insulation workers were caused by asbestosis. Pulmonary fibrosis sufficient to interfere with respiratory or cardiovascular function can be prevented by reducing asbestos dust concentration to levels that are still far above any likely to be encountered in community air. Calcified pleural plaques occur frequently in workers exposed to asbestos.

On the basis of sufficient evidence of carcinogenicity in humans, asbestos and all commercial forms of asbestos are now recognized as "known to be human carcinogens."[16] Occupational exposure to chrysotile, amosite, anthophyllite, and mixtures containing crocidolite has resulted in a high incidence of lung cancer. Mesotheliomas (a neoplasm derived from cells lining the chest or abdominal cavities) have been observed after occupational exposure to crocidolite, amosite, and chrysotile asbestos. Gastrointestinal cancers occurred at an increased incidence in groups occupationally exposed to several types of asbestos. An excess of laryngeal cancer has also been observed in some groups of exposed workers. No clear excess of cancer has been associated with the presence of asbestos fibers in drinking water. Mesotheliomas have occurred in

individuals living in the neighborhood of asbestos factories and mines and in people living with asbestos workers.[16] As mentioned in Section 5.6.1, there is synergism between cigarette smoking and asbestos exposure in relation to lung cancer development.

It is widely recognized that problems associated with asbestos still exist, although they have greatly decreased in the U.S. The health problems associated with asbestos exposure are well known and widely recognized. As a result, occupational standards have become more stringent, and testing methods more sophisticated. With improvement in remediation methods, and the availability of more information about how asbestos fibers cause health problems in humans, the threat of asbestos to humans should continue to diminish.

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9.9 REVIEW QUESTIONS

1. Define ''primary particulates'' and ''secondary particulates.''
2. Explain the characteristics of particulate matter.
3. Why is size particularly important in determining the toxicity of particulate matter?
4. What are some of the natural and anthropogenic sources of particulate matter?
5. Explain the importance of H_2SO_2 as particulate matter.
6. Explain the reasons for the toxicity of particulate matter.
7. What is pneumonoconiosis?
8. What is silicosis? Why is silicosis important in public health and toxicology?
9. Explain the current understanding of the mode of action for silicosis.
10. Explain why combustion of coal is considered the largest source of environmental Be contamination.
11. How is Be related to DNA?
12. What is ATA? Explain how it may help alleviate Be toxicity.
13. List the health effect of asbestos.
14. What types of cancers are associated with asbestos exposure?
15. How are smoking and asbestos exposure related?

Chapter 10

Environmental Fluoride

10.1 INTRODUCTION

Fluorine is the lightest element in Group VII of the periodic table, with the atomic number 9 and the atomic weight of 18.998. It has a single isotope, and its valence in all naturally occurring compounds is one. Although fluoride (F) is not listed as one of the "Criteria Air Pollutants" regulated by U.S. Environmental Protection Agency, it is nevertheless a very important gaseous air pollutant. Indeed, F is the most phytotoxic air pollutant because it can damage plants at extremely low concentrations. Additionally, adverse effects are not limited to those caused by airborne F: high levels of waterborne F are also hazardous to both human and animal health. For example, in China and India millions of people are suffering from dental and skeletal fluorosis – abnormal or poisoned tooth and bone conditions induced by F – mainly due to consumption of high levels of F in drinking water.

This chapter begins with an introduction to the sources and forms of F found in the environment, and then discusses the way in which F influences the health of plants, animals, and humans. Whenever applicable, reference will be made to the growing concerns shared by several less-developed countries that are experiencing significant growth in their economies, such as China and India.

10.2 OCCURRENCE AND FORMS OF FLUORIDE

10.2.1 Introduction

Fluoride is ubiquitous: it rarely occurs free in nature, but combines with a variety of elements to form fluorides that exist in minute amounts in air, water, minerals and soils, foods, and body tissues. Fluoride found in the environment is derived from both natural and anthropogenic sources. Natural sources of F include volcanism, aerosols from ocean spray, and soil particles blown into the atmosphere, etc., while anthropogenic sources are found mostly in industrial facilities. Fluoride emitted into the atmosphere from these sources consists of both gaseous and particulate forms, and as such can contribute F to surface waters.

10.2.2 Air

Fluoride concentrations in the air in U.S. residential and rural communities vary markedly, ranging from less than 0.04 ppb to 1.2 ppb (0.03 to 0.90 μg/m^3).[1] In many cities in less-developed countries, the level is much higher. For example, in Beijing, China, the level is reported to be 0.11 to 2.14 ppb (0.08 to 1.61 μg/m^3; average, 0.61 μg/m^3).[2]

10.2.3 Natural Waters

Fluoride content in natural waters in the U.S. ranges from 0.02 to 0.2 ppm. In 1969, there were 2630 communities in the U.S. with a drinking water supply that contained a natural F concentration of 0.7 ppm or more (Figure 10.1).[3] River waters contain 0.0 to 6.5 ppm, with an average of 0.2 ppm. Groundwaters contain from 0.1 to 8.7 ppm, depending on the rocks from which the waters flow. Fluoride level in seawater is about 1.4 ppm.[1]

10.2.4 Minerals and Soils

The F content in rocks is typically about 0.06 to 0.09% (by weight). The most important F-containing minerals are fluorspar or fluorite (CaF_2), cryolite (Na_3AlF_6), and fluorapatite ($Ca_{10}F_2[PO_4]_6$).[1] Whenever any of these minerals

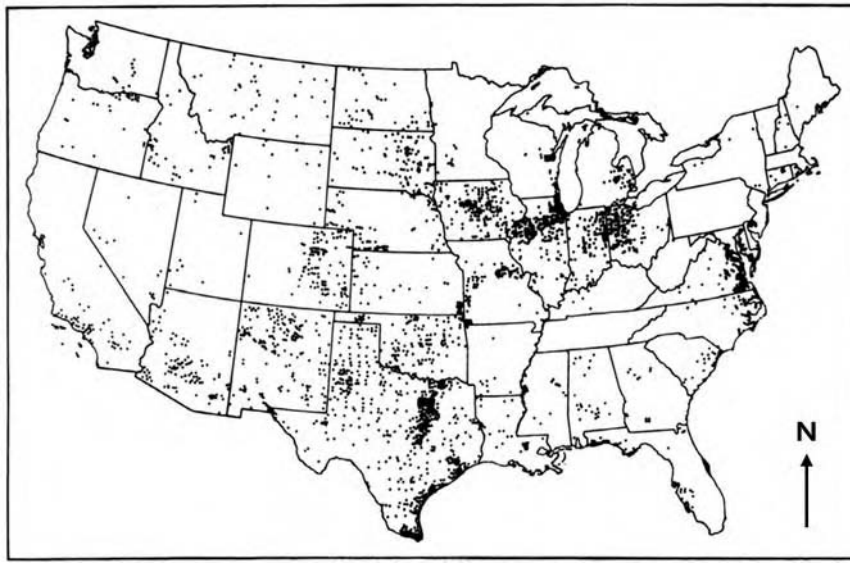


Figure 10.1 Distribution of communities in the U.S. with natural fluoride concentrations of 0.7 ppm or more in community water supplies.
Source: adapted from NAS Subcommittee on Fluorosis, *Effects of Fluorides in Animals*, National Academy of Sciences, 1974.

are used in industrial processes, some amounts are emitted into the environment as gases or particulates. These, in turn, are precipitated onto the ground and subsequently absorbed in soils. The absorbed F may assume different forms, depending on such factors as soil pH, organic matter and clay content, and exchangeable calcium (Ca) content.

10.2.5 Foods

Because F is ubiquitous, virtually all foods contain trace amounts of the mineral. Fluoride-containing foods and beverages are, therefore, the most important sources of F intake. F intake from food and beverages by a male residing in a fluoridated community in the U.S. is about 1 to 3 mg/day. It is decreased to $\leq$1.0 mg/day in a nonfluoridated area.[4] The intake from drinking water ranges from 0.1 to 0.5 mg/day in nonfluoridated communities, whereas in fluoridated communities it may amount to 1 to 2 mg/day. Total F intakes in several countries are much higher than those in the U.S. For instance, in the West Midlands, the region of the U.K. with the longest history of fluoridation, a large number of residents were reported to take in 3 mg F or more per day.[5]

Plants can absorb F from soil, water, or the atmosphere. F contents in plants range from 0.1 to 10 ppm (dry basis), depending on the species. Several plant species are known as F accumulators. Examples include camellia (620 ppm), tea (leaves, 760 ppm), and elderberry (3600 ppm, dry basis). It is interesting to note that although tea leaves are an important F-accumulator, tea beverage may contain less than 0.5 mg F per cup. Table 10.1 lists the F content of several varieties of foods produced in U.S.

Table 10.1 Fluoride Content of Selected Food

Food	F content (ppm on dry basis)
Milk	0.04–0.55
Meats	0.01–7.7
Fish	0.10–24
Cheese	0.13–1.62
Butter	0.4–1.50
Rice and peas	10
Cereal and cereal products	0.10–0.20
Vegetables and tubers	0.10–2.05
Citrus fruits	0.04–0.36
Sugar	0.10–0.32
Coffee	0.2–1.6
Tea	
infusion	0.1–2.0
instant (solution)	0.2

Source: adapted from NAS/NRC Committee on Biologic Effects of Atmospheric Pollutants, *Fluorides*, National Academy of Sciences, 1971.

10.3 INDUSTRIAL SOURCES OF FLUORIDE POLLUTION

10.3.1 Introduction

Anthropogenic sources of F include a variety of industries, such as primary aluminum production, phosphate fertilizer and elemental phosphorus (P) plants, primary iron and steel production, and ceramics industries (tile, brick and glass works, etc.). Combustion of fuel, especially coal, and incineration of solid waste also cause F emission. F content in coal ranges from 0.001 to 0.048% in the U.S. (average, 0.008%). The forms of F that are emitted from industrial processes include hydrogen fluoride (HF), fluorspar, cryolite, and silicon tetrafluoride (SiF_4). In addition to deposition in surface waters, airborne F may be deposited onto the ground and taken up by soils, plants, and animals (Figure 10.2).

Representative industrial sources of F emission are discussed in the following sections.

10.3.2 Manufacture of Phosphate Fertilizers

The starting material for manufacture of normal superphosphate fertilizer is phosphate rock, composed of mainly fluorapatite. In this process, fluorapatite reacts with sulfuric acid (H_2SO_4) and water, producing $CaH_4(PO_4)_2$. The overall chemical reaction for the manufacture is shown in Reaction 10.1. As the F content of the ore is approximately 3%, a substantial quantity of HF is

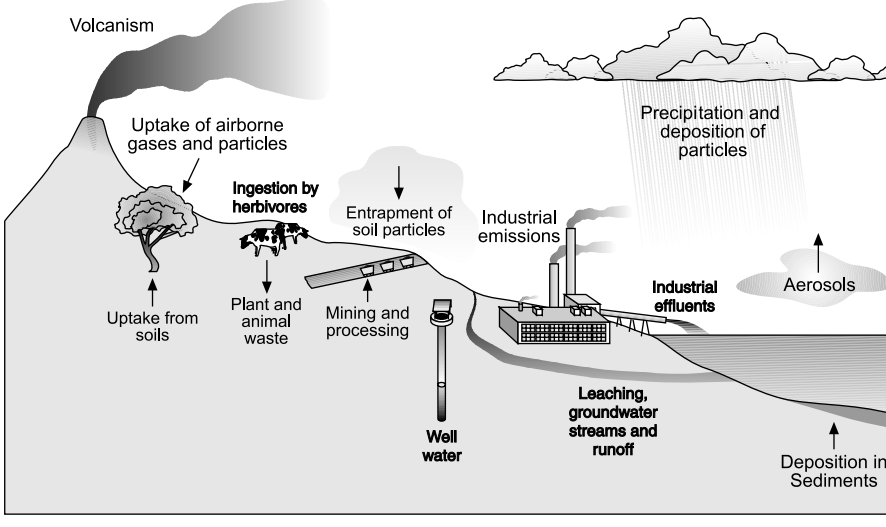


Figure 10.2 Environmental transfer of fluoride.
Source: adapted from NAS/NRC Committee on Biologic Effects of Atmospheric Pollutants, *Fluorides*, National Academy of Sciences, 1971.

produced. HF reacts with silicon oxide (SiO_2) in the fluorapatite to form SiF_4 gas, as shown in Reaction 10.2.

$$Ca_{10}F_2(PO_4)_6 + 7H_2SO_4 + 3H_2O \rightarrow 3CaH_4(PO_4)_2.H_2O + 7CaSO_4 + 2HF \uparrow \quad (10.1)$$

Fluorapatite

$$SiO_2 + 4HF \rightarrow SiF_4 + 2H_2O \quad (10.2)$$

In the aqueous scrubber, SiF_4 readily reacts with water, forming fluorosilicic acid (H_2SiF_6), as shown in Reaction 10.3. Fluorosilicic acid is highly soluble in water and is readily absorbed by plants.[1]

$$3SiF_4 + 2H_2O \rightarrow SiO_2 + 2H_2SiF_6 \quad (10.3)$$

10.3.3 Manufacture of Aluminum

Manufacture of aluminum is carried out almost exclusively by the Hall–Herouet process, where alumina (Al_2O_3) is dissolved in molton cryolite and reduced electrolytically. The electrolytic cell contains a carbon lining, serving as both the cathode and the container for the melt. Reaction 10.4 shows the process.

$$Al_2O_3 + 2C \xrightarrow{\text{Catalysts}} 2Al + CO + CO_2 \quad (10.4)$$

As shown in Reaction 10.4, CO and CO_2 are the two gases emitted in the process: there is no emission of any F-containing substance. In reality, however, a number of other substances are emitted in the process. This is because several catalysts, including CaF_2, AlF_3, and cryolite, are used in the eletrolysis of alumina, and as these are heated at high temperatures, some will escape from the cells, contaminating the surrounding atmosphere. In addition to CO and CO_2, several other gases, including SO_2, SiF_4, HF, COS, CS_2, He, and water vapor, are also produced in the electrolysis cells, and are emitted into the surrounding air. Moreover, a large number of particulates are also emitted, including Al_2O_3, carbon (C), cryolite, AlF_3, CaF_2, Fe_2O_3, and chiolite ($Na_5Al_3F_{14}$).

10.3.4 Manufacture of Steel

In the manufacture of steel, calcium fluoride (CaF_2) is used as a flux (a substance that promotes fusion of metals) in open-hearth smelters to increase fluidity of the slag and enhance the removal of impurities, such as P and sulfur (S), from the melts. F compounds emitted from this operation include HF and CaF_2.[1]

10.3.5 Combustion of Coal

As mentioned previously, coals mined in U.S. contain about 0.001 to 0.048% F, usually as fluorapatite or fluorspar. Combustion of coal in power plants, therefore, emits considerable quantities of F into the atmosphere. During combustion, about half of the F in coal is emitted as gaseous HF and SiF_4 and particulate matter.

The dramatic increase in the use of coal as an energy source in many cities and areas in the world has caused atmospheric F-pollution to increase steadily. This trend is especially conspicuous in a number of less-developed countries. For example, studies show that several cities in China, including Chongqing and Beijing, are experiencing severe fluoride air pollution problems arising mostly from coal combustion.[2,6]

In Beijing, coal is the dominant energy source, accounting for more than 75% of the total energy consumption. Combustion of coal for heating in winter accounts for 23% of the annual coal combustion. Furthermore, the F content of coal consumed in the city is reported to be 163 μg/g, more than double the mean value of 80 μg/g for coals of other parts of the world.[2] Another important source of F in Beijing is dust from fresh concrete used for construction. Factors such as these have contributed to the elevated F concentrations of wet depositions in the city. For example, the annual volume-weighted average concentration of soluble F of ambient aerosol is reportedly 60 $\mu g/m^3$, which is 75 times higher than the concentration observed in the air sample taken in the city of Morioka, a city without fluoride pollution, in northern part of Japan.[6]

Fluoride has also been traced to runoff from application of insecticides and herbicides. In addition to direct runoff into surface waters, airborne F may be deposited into surface water and onto the ground, and eventually taken up by soils, plants, and animals (Figure 10.2).

10.4 EFFECTS ON PLANTS

HF is the most phytotoxic air pollutant. The high toxicity of F and its compounds is due to their rapid absorption and the inherent toxicity of the element. F can cause injury to susceptible plants at concentrations below 1 ppb (0.8 $\mu g/m^3$) for exposure periods of 7 days or less.[7,8,9] Exposure to F can result in marked increases in foliage F levels. The extent of increases depends upon factors such as duration of exposure, atmospheric F levels, and species or variety of plants. F-induced effects in plants may be viewed based on four levels of biologic organization: cellular, tissue or organ, organism, and ecosystem (Table 10.2).[1]

F accumulates in plant leaves mainly as a result of diffusion from the atmosphere through the stomata or through absorption from soil by root. In contrast to other major air pollutants, such as sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and ozone (O_3), discussed in Chapter 8, F accumulates in the

Table 10.2 Nature of Fluoride-Induced Effects in Plants at Four Levels of Biologic Organization

Cellular	Tissue	Organism	Ecosystem
Effects on enzymes and metabolites			Increased F in ecosystem
	Decreased assimilation Altered respiration		
Modification of celorganelles and metabolism	Altered growth and development	Modified growth	Increased F burden of animals
Pathway disruption	Chlorotic lesions	Reduced reproduction	Fluorosis in animals
Cellular modification		Decreased fitness for environment	
	Necrotic lesions		
Disruption and death of cell	Death or abscission of leaf	Death of plants	Desolation

Source: adapted from NAS/NRC Committee on Biologic Effects of Atmospheric Pollutants, *Fluorides*, National Academy of Sciences, 1971.

foliage of plants. The plants then serve as a vehicle for transfer of F to herbivores, with the potential for inducing dental and skeletal fluorosis.

F induces both structural and functional changes in plant cells. Changes occur in cellular and subcellular membranes, causing subsequent injuries. Although plants differ widely in their susceptibility to F injury, accumulation of high levels of F in leaves normally leads to chlorosis and necrosis. Chlorosis is associated with lowered chlorophyll content in the leaf and thus leads to lowered photosynthesis. Similarly, the destruction of part of the leaf, resulting from necrosis, will cause a comparable reduction in photosynthesis. Both chlorosis and necrosis lead to reduced growth and yield. Tree death can result when the injuries are severe (Figure 10.3a). Contrary to NO_2 or SO_2, F induces damage in leaf tips and margins of many plant species (Figure 10.3b and Figure 10.3c).

During the past several decades, numerous field and laboratory studies have been conducted on the phytotoxicity of F throughout the world.[7–15] In laboratory experiments, plants exposed to various concentrations of F generally exhibit concentration-dependent growth impairment. For example, mung bean seedlings exposed to 0, 0.1, 1.0, and 5.0 m*M* F (as NaF) for 24, 48, and 72 hours showed inhibition of germination (Figure 10.4). Similar observations have frequently been made in field studies. An example is presented in Case Study 10.1.

Case Study 10.1

In 1979, researchers in the northern part of Taiwan observed a previously unknown foliage disease of rice plants. Leaves of the plants, grown in an area adjacent to ceramic and brick-making industrial facilities, manifested acute

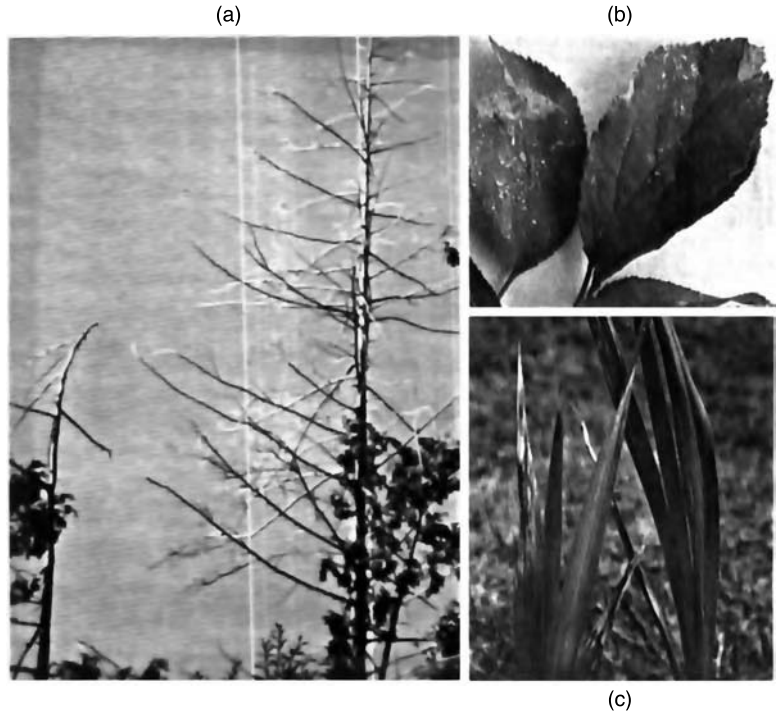


Figure 10.3 Fluoride-induced injuries to plants: (a) fir tree growing in an area adjacent to an aluminum manufacturing plant (analysis of needles from adjacent trees showed F levels at approximately 500 ppm, on dry weight basis), (b) pear leaves with tip and marginal necrosis, and (c) gladiolus leaves with tip necrosis.

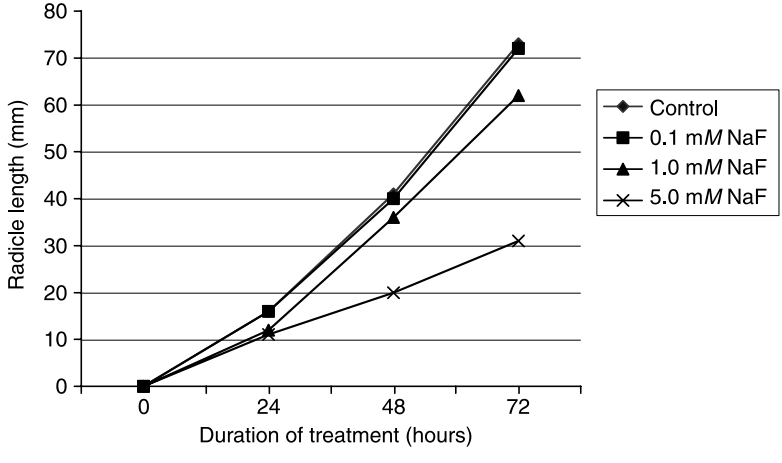


Figure 10.4 Effect of NaF on mung bean germination.
Source: Yu, M.-H., unpublished data, 2003.

symptoms of chlorosis and tip necrosis. Studies in 1983 showed that ambient F concentration in the area range from 0.4 to 15 μg/kg (average, 4.5 μg/kg). Analysis of the F content of leaves of rice plants grown in the area revealed marked increases, and the levels were correlated with increasing severity of leaf injury. The severely injured leaves contained 80 times as much F as the healthy leaves. Subsequently, laboratory experiments involving fumigation of rice seedlings with HF were conducted. The results showed leaf symptoms that were similar to those observed in the field. These observations suggested to the researchers that F emitted from the ceramic and brick factories was the cause of the observed "new rice disease."[16]

10.5 EFFECTS ON ANIMALS

10.5.1 Introduction

Animals normally ingest small amounts of F in their rations without observable adverse effects, but excessive intake can be detrimental. Common sources of excessive F intake include: forages subjected to airborne contamination or grown in soils containing high F levels, water containing high levels of F, and feed supplement containing high levels of F. The effect of F on domestic animals may be acute or chronic, depending on the levels to which animals were exposed.

10.5.2 Acute Effects

F and arsenic (As) have caused detrimental effects on livestock in the U.S. and several other industrialized countries. The sources of F pollution are limited mostly to phosphate-fertilizer manufacturing, aluminum production, fluorohydrocarbons, and heavy-metal production. A safe level of soluble F in animal rations ranges from 30 to 50 mg/kg for cattle and from 70 to 100 mg/kg for sheep and swine. F poisoning may cause physiological effects, such as gastroenteritis, muscular weakness, pulmonary congestion, nausea, vomiting, diarrhea, chronic convulsions, necrosis of mucosa of the digestive tract, anorexia, cramping, collapse, and respiratory and cardiac failure, possibly leading to death.

10.5.3 Chronic Effects

The two most conspicuous and thoroughly studied manifestations of chronic F poisoning are dental and skeletal fluorosis. Once absorbed into animal body, F has a great affinity for developing and mineralizing teeth. This affinity can either enhance tooth development or induce dental lesions, depending on the amounts of fluorides ingested. Dental fluorosis is the first sign of chronic F toxicity. It is exhibited by white, yellow, brown, and black discoloration of tooth enamel, either in spots or in horizontal streaks. An affected tooth is also

subject to more rapid wear, and erosion of the enamel from the dentin. It is noteworthy that dental lesions are not seen in animals brought into endemic fluorosis areas after their permanent teeth have erupted.[17]

Recent studies indicate a widespread chronic effect of environmental F on wildlife. Fluoride contamination of vegetation arises from various industrial activities, noted previously, and combustion of coal. Studies conducted by European scientists have shown that a large number of deer in several European countries suffer from both dental and skeletal fluorosis.[18,19] However, during the past three decades, much improvement has been made in controlling F emission, as evidenced by comparative field studies showing that F contamination of vegetation has decreased significantly in recent years. Concomitantly, decreases were observed in F levels in dental and skeletal samples of wild animals.

Studies on deer population affected by F are relatively limited in the U.S. There is no obvious active research in this area, presumably because atmospheric F pollution in the U.S is limited to local areas, and is not considered a serious environmental problem. Nevertheless, studies have demonstrated increased bone F levels in other animals found around F-emitting industrial facilities. Furthermore, it is often found that F levels are inversely related to the distance between the industrial facilities and the site of animal collection. For example, the mean F levels in bones of deer mice, collected from various sites from around an aluminum plant in North America, were found to be 1724 ±142 mg/kg and 237±89 mg/kg dry weight for animals collected at 2 km and 32 km from the plant, respectively (Figure 10.5).[20]

The impact of airborne F on wildlife is also demonstrated in the teeth of black-tailed deer. Figure 10.6 shows a comparison between dental disfigurement and abnormal tooth wear pattern of a female black-tailed deer (deer A) (Figure 10.6a) and normal tooth wear pattern of a male black-tailed deer (deer B) (Figure 10.6b). Deer A was killed on road near an aluminum manufacturing plant, whereas deer B was killed on road in an area with no industrial facilities.

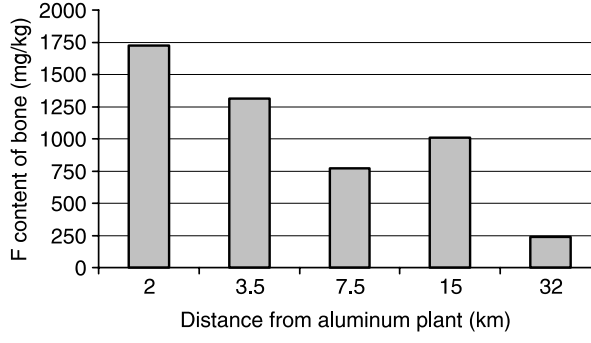


Figure 10.5 Relationship between fluoride levels in bones of deer mice and distance of collection sites from an aluminum plant.
Source: Yu, M.-H., unpublished data, 2003.

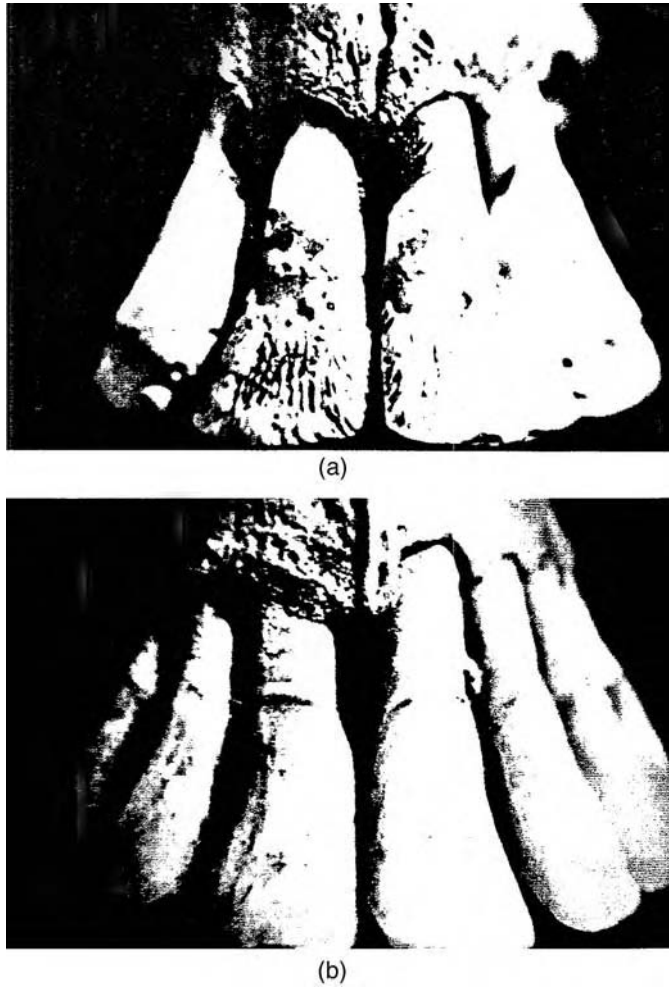
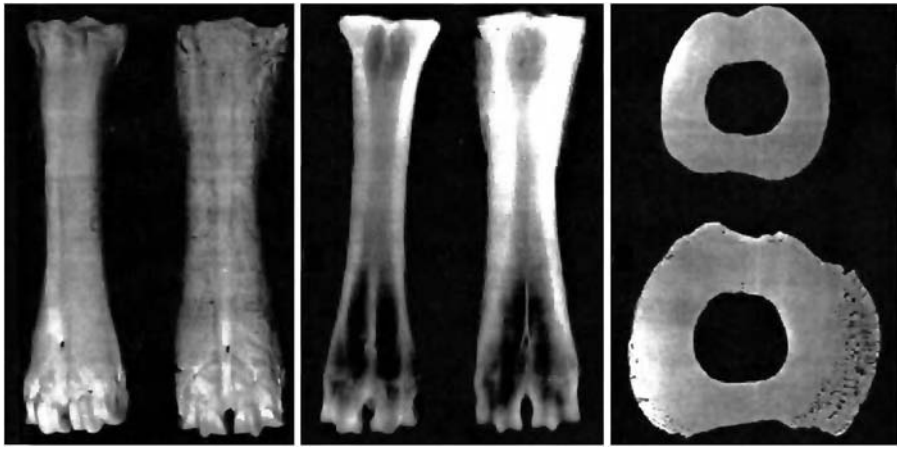


Figure 10.6 Impact of airborne fluoride on teeth of black-tailed deer: (a) dental disfigurement and abnormal tooth wear patterns in a deer killed in an area adjacent to an aluminum plant (female, ca. 1.5 years old), and (b) normal tooth wear patterns of a deer killed in an area with no industrial facilities (male, ca. 2.5 years old).
Source: Newman, J.R. and Yu, M.-H., *J. Wildlife Dis.*, 12, 39, 1976.

Analysis of the F content in bone samples from these two animals showed that F levels of the bones from deer A were 15 to 20 times higher than those of the bones from deer B.[21]

In addition to inducing tooth mottling, F can also cause skeletal fluorosis. Skeletal fluorosis causes affected bones to lose their normal, hard, smooth luster and appear rough, porous, and chalky white. A generalized hyperostosis (excessive formation of bone tissue, especially in the skull) and, in some cases, exostotic lesions of the otherwise smooth, long bones may be observed (exostosis is a spur or bony outgrowth from a bone) (Figure 10.7). Lameness or stiffness is an intermittent sign of F toxicity. Figure 10.8 shows a lame cow,



(a) (b) (c)

Figure 10.7 Bones from dairy cows with skeletal fluorosis. (a) Left: metatarsal bone from a dairy cow fed 12 ppm F from 3 or 4 months to 7.5 years of age – the bone is normal. Right: metatarsal bone from a dairy cow fed 93 ppm F for the same period – the bone shows marked periosteal hyperostosis with a roughened surface. (b) Radiographic comparison of the bones in (a). (c) Upper: cross section of a metatarsal bone from a dairy cow fed 12 ppm F from 3 or 4 months to 7.5 years of age – the bone is normal. Lower: cross section of a metatarsal bone from cow ingesting 93 ppm F for the same period – the bone shows definite osteofluorosis.
Source: adapted from Greenwood et al. *Fluorosis in Cattle*. Special Report 17. Agricultural Experiment Station, Utah State University, Logan, UT, 1964, p.36.

Figure 10.8 A lame cow suffering from F toxicity.
Source: Yu, M.-H. (Personal communication, 2004.)

Table 10.3 Fluoride Tolerances in Diets of Livestock

	F tolerance in diet (ppm)	
	Breeding or lactating animals	**Finishing animals**
Dairy and beef heiffers	30	100
Dairy cows	30	100
Beef cows	40	100
Sheep	50	160
Horses	60	–
Swine	70	–
Turkeys	–	00
Chicken	150	–

Source: adapted from NAS/NRC Committee on Biologic Effects of Atmospheric Pollutants, *Fluorides*, National Academy of Sciences, 1971.

suffering from chronic exposure to F through feed containing high F levels. The clinical basis for the lameness is not well understood.[1]

Impaired appetite is normally observed, which may result in decreased weight gain, cachexia, and lower milk yield. Decrease in milk production may be secondary to appetite impairment or other responses. Evidence that animals may be suffering chronic F effects may be obtained from chemical analysis of the feed, or by testing for elevated levels of F in urine and body tissues.[22] Affected animals may exhibit increased susceptibility to other environmental stresses and decrease in longevity.

A number of factors influence the manifestation of dental and skeletal fluorosis, including:

- amount and bioavailability of F ingested
- duration of ingestion
- species of animals involved (Table 10.3)
- age at time of F ingestion
- mode of F exposure (e.g., continuous or intermittent)
- individual biologic response
- presence of synergistic or antagonistic substances
- nutritional and general health status of animals
- presence of other stress factors, such as those caused by poor management

10.6 EFFECTS ON HUMANS

10.6.1 Daily Intake

Daily intake of F by individuals in the U.S. is about 0.2 to 0.3 mg from food, 0.1 to 0.5 mg from water (1 to 2 mg if water is fluoridated), and varying quantities from beverages (F content of wine is 0 to 6.3 ppm, beer contains 0.15 to 0.86 ppm, and milk, 0.04 to 0.55 ppm). The amount of F inhaled from air is about 0.05 mg/day.

10.6.2 Absorption

Absorption of F from the gastrointestinal tract occurs through a passive process; it does not involve active transport.[1] Absorption is rapid and probably occurs in the lumen. The rate of absorption is dependent on the F compounds involved, e.g., 97% of NaF, 87% of $Ca_{10}F_2(PO_4)_6$, 77% Na_3AlF_6, and 62% CaF_2 are absorbed. About 50% of the absorbed F is excreted by the kidneys while the remainder is stored, primarily in calcified tissues. No significant F accumulation occurs in soft tissues.

Bone has a great affinity for F and incorporates it into hydroxyapatite ($Ca_{10}(OH)_2(PO_4)_6$), forming fluorapatite ($Ca_{10}F_2(PO_4)_6$). Even at low levels of F intake, appreciable levels of F will, in time, accumulate in calcified tissues. The effectiveness of low levels of F intake in reducing dental caries in humans, rats, and some other species of animals has been reported. In human population, water supply containing 1ppm F is reported to reduce by more than 50% dental caries incidence in individuals who consume F from infancy. Fluoride is incorporated into tooth mineral as fluorapatite at the time of calcification.

10.6.3 Acute Effects

Exposure to high levels of F results in varying degrees of injuries (see for example Case Study 10.2). The lethal dose of inorganic F has been estimated to be in the range of 2.5 to 5 g for a 70 kg male, or approximately 50 mg/kg, a dose similar to the LD_{50} for several animal species. The cause of death is probably related to the prompt binding of serum Ca and magnesium (Mg) by F. Clinical symptoms include excessive salivation, perspiration, vomiting, painful spasms of limbs, stiffness, nausea, chronic convulsion, necrosis of the mucosa of the digestive tract, and heart failure.

Case Study 10.2

A fluoride overfeed occurred in 2002 at a well site near an elementary school in Portage, Michigan.[22] The incident resulted in a high F concentration (92 mg/l) in the drinking water at the school. Several students who drank water from the school fountain reportedly suffered nausea and vomiting. Toxicological studies were conducted to assess the risk. Based on the symptoms experienced by the students, it was concluded that the F had irritated the stomach, causing the observed symptoms, but that no appreciable long-term adverse health effects would occur.[23]

10.6.4 Chronic Effects

Fluoride accumulates in the skeleton during prolonged, high-level exposures. Radiological evidence shows hypermineralization (osteofluorosis) occurs when bone F concentrations reach about 5000 ppm.[24] Coupled with other environ-

mental factors, such as poor nutrition and health status, patients may suffer severe skeletal dysfunction. Vomiting and neurological complaints have also been observed in some patients. Increased levels of F in serum and urine usually occur. Fluoride exposure leads to cell damage and induces necrosis. Eventually, F produces massive impairment in the functions of vital organs, particularly when given orally.

In some parts of the world, such as India, Mexico, and China, the water supplies in many villages (usually from wells) contain high levels of F, in some cases higher than 20 ppm. As a result, osteofluorosis is common. Published reports indicate that in China approximately 20 million people may be afflicted by chronic F poisoning.[25] As mentioned previously, the dramatic increase in the use of coal as an energy source has resulted in many parts of China suffering from rising emissions of F into the environment. Research conducted by Ando et al.[6] showed high incidences of dental and skeletal fluorosis in some rural areas in China.

Dental fluorosis is the first sign of chronic F toxicity. It is manifested by white, yellow, brown, and black discoloration of tooth enamel, either in spots or in horizontal streaks. One of the earliest symptoms of dental fluorosis is mottled enamel.

Skeletal fluorosis refers to accumulation of F in skeletal tissues and is associated with pathological bone formation. It is one of the most severe effects of F on humans, and is caused by intake of elevated levels of F over a long period. In the F-afflicted areas studied by Ando et al.,[6] combustion of coal and coal bricks was found to be the primary source of gaseous and aerosol F in the human environment. Airborne F from the combustion of coal was found to pollute extensively both the living environment and the food, such as corn, chilies, and potatoes, that the residents consume.

Several groups of researchers have reported reproductive effects of F in humans.[26,27] Ortiz-Perez et al.[28] recently studied the reproductive parameters in Mexican residents. Two groups of male residents were identified: the high-fluoride-exposed group (HFEG), those exposed to F at 3 to 27 mg/day, and the low-fluoride-exposed group (LFEG), exposed to 2 to 13 mg/day. Increased urinary F levels (3.2 mg/g creatinine vs. 1.6 mg/g creatinine) were found in the HFEG, compared with the LFEG. Levels of reproductive hormones were also measured; the HFEG showed higher follicle stimulating hormone but lower inhibibin-B, prolactin, and free testosterone serum concentrations than the LFEG, while no differences were found for total testosterone, estradiol, or lutenizing hormone between the two groups.[28]

10.7 BIOCHEMICAL EFFECT

10.7.1 In Plants

Fluoride is widely known to be a metabolic inhibitor. In plants, F affects many biological processes, including glycolysis, Krebs-cycle reactions, photosynth-

esis,[7,8] protein synthesis, lipid metabolism, and others. Much of the action of F on these processes can be attributed to F-dependent inhibition of enzymes. Enzymes that are inhibited by F include enolase, phosphoglucomutase, phosphatase, hexokinase, PEP carboxylase, pyruvate kinase, succinic dehydrogenase, malic dehydrogenase, pyrophosphatase, phytase, nitrate reductase, mitochondrial ATPase, and urease.[12]

Inhibition of lipase,[13] amylase (Table 10.4),[14] and invertase[15] activities *in vivo* has been observed in germinating mung bean seedlings exposed to NaF at concentrations of 1 m*M* and above. Fluoride-induced inhibition of amylase and invertase appears to involve the removal of cofactor Ca^{2+} by F^{-}. In a separate study, Narita et al.[29] showed that inhibition of [2-^{14}C]thymidine incorporation into DNA occurs in mung bean seedlings exposed to 1 m*M* NaF for 24 hours and above (Figure 10.9). The inhibition suggests a concomitant influence on protein synthesis.

The inhibition of plant enzymes such as these is often reflected by compositional changes in tissues. For example, soybean leaves exposed to 30 ppb of HF exhibited lowered sucrose content, while the levels of both glucose and fructose were elevated.[14] Marked increases in several organic acids also occur, including malic, malonic, succinic, and citric acids.[13] The inhibition of amylase[11] and invertase[12] in germinating mung bean seedling exposed to NaF is often accompanied by increased sucrose levels in the root.

While it is clear that the action of F on metabolism is complex and involves a variety of enzymes, the mode of action of fluoride ions on these enzymes is not so clear. Nevertheless, the principal mechanisms that have been suggested include:

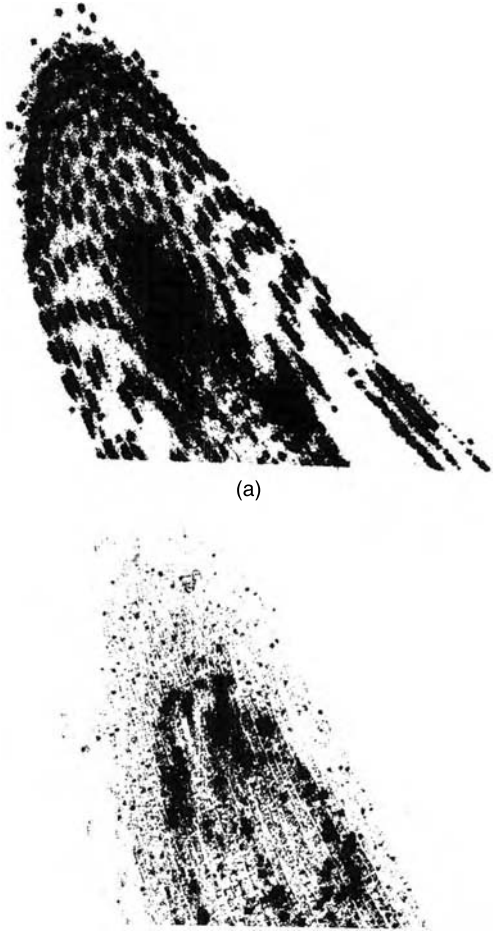
- formation of complexes with metalloenzymes
- removal of a metal cofactor, such as Ca or Mg, from an enzyme system
- binding to the free enzyme or to the enzyme substrate complex[9]
- disruption of hydrogen bonds on protein molecules[15]

Because hydrogen bonding is important in the maintenance of the tertiary structure of protein molecules, disruption of an enzyme protein by F would lead to enzyme inhibition.

Table 10.4 Effects of NaF on α-Amylase from Mung Bean Cotyledon

	Specific activity (nmol/mg per minute)			
NaF (m*M*)	**48 hours**	**Percent of control**	**72 hours**	**Percent of control**
0 (control)	13.1	100	27.8	100
0.1	14.6	111	24.4	88
1.0	11.3	86	24.2	87
5.0	8.3	63	22.2	80

Source: Yu, M.-H., Shumway, M.O. and Brockbank, A., *J. Fluorine Chem.*, 41, 95, 1988.



(a)

(b)

Figure 10.9 Autoradiographs showing [2-^{14}C]thymidine incorporation in radicle of mung bean seedling treated with (a) water or with (b) 1 m*M* NaF for 12 hours. (Black grains show the developed silver deposited in the nuclei of dividing cells of the radicle, and are considered the sites of [2-^{14}C]thymidine incorporation into DNA in the tissue.
Source: Narita, A. et al., *Fluoride*, 29, 72, 1996.

As noted in Chapter 6, superoxide dismutase (SOD) is an important antioxidant enzyme. Field and laboratory studies have shown that SOD activities in different plant tissues exposed to F were either enhanced or lowered. For instance, mung bean seedlings exposed to 0.2 m*M* NaF showed an enhanced SOD activity, whereas exposure to 1 m*M* NaF and above resulted in depressed SOD activity.[30]

10.7.2 In Animals and Humans

F inhibits the metabolism of carbohydrates, lipids, and proteins. In animals and humans, a large number of enzymes are depressed by F, including enolase,

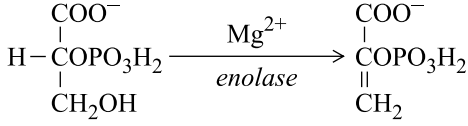
ATPase, lipase, and cholinesterase. Inhibition of glycolysis, due in part to decreased enolase activity, may be responsible for the hyperglycemia observed in laboratory animals exposed to F.

F stimulates adenylcyclase activity in all tissues so far examined (adenylcyclase catalyzes the formation of cyclic AMP [cAMP] from ATP). F also affects functions controlled by Ca in humans, as it does in plants. These functions include blood clotting, membrane permeability, and cholinesterase activity. Fluoride inhibition of reactions involving Ca is generally attributed to the formation of CaF_2, as shown in Reaction 10.5 and Reaction 10.6, below:

$$[\text{Protein-Ca}] \rightleftharpoons \text{Protein} + Ca^{2+} \tag{10.5}$$

$$Ca^{2+} + {}^{2}F^{-} \rightleftharpoons [CaF]^{+} + F^{-} \rightleftharpoons CaF_2 \tag{10.6}$$

Enzyme systems requiring Mg are also mediated by F. For example, F has been shown to inhibit enolase, a Mg-requiring enzyme responsible for the conversion of 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway (Reaction 10.7). According to some researchers, the inhibition results from the formation of a magnesium-fluoro-phosphate complex, thus essentially making Mg unavailable for the enzyme.



(10.7)

2-phosphoglycerate phosphoenolpyruvate

The inhibition of myosin ATPase by F is another example of F interacting with Mg. Energy transduction in myosin converts the chemical energy released by ATP into mechanical work at the site of force generation. Myosin is a fibrous globulin that interacts with actin (a protein in muscle that is active in muscular contraction) and ATP, with resulting enzymatic hydrolysis of ATP to ADP and inorganic phosphate (P_i):

$$\text{ATP} \rightarrow \text{ADP} + P_i \tag{10.8}$$

During hydrolysis of ATP, myosin subfragment 1(S1) requires the presence of an Mg^{2+} ion to stabilize a nucleotide or nucleotide analog in the active site of S1. In the presence of F, Mg^{2+} and MgADP form a complex MgADP–MgFx that traps the active site of S1 and inhibits myosin ATPase.[31]

As previously mentioned, F is shown to inhibit protective enzymes, such as SOD, glutathione peroxidase (GSHPx), and catalase, in various human tissues. Inhibition of one or more of these enzymes may allow free-radical-induced reactions to occur, leading to cellular and tissue damages.

10.8 NUTRITIONAL FACTORS AFFECTING FLUORIDE TOXICITY

There is a growing interest in the interaction of nutrition with F toxicity as several nutrients have been shown to alleviate injuries caused by exposure to F. The nutrients studied so far include proteins and Ca, and vitamins C (ascorbic acid), D, and E. Glutathione (GSH), which is not a nutrient but a well-known antioxidant, has also been studied. The adverse effects of F are often mitigated by administration of one or more of these nutrients.

A large number of studies have indicated a relationship between vitamin C and F-exposure in animals. In one of these studies, growing chicks (*Gallus domesticus*) were fed a diet supplemented with 150 ppm of F as NaF.[32] At the end of 4 weeks, no differences in body weight were observed; however F-treated chicks showed a marked decrease in ascorbic acid levels in the heart, spleen, brain, gizzard, and pancreas, while the levels were increased in the lungs and kidneys. In a separate study, growing cockerels were given a diet supplemented with 150, 300, or 500 ppm F for 4 or 8 weeks, and then subjected to analysis of tissue levels of ascorbic acid and dehydroascorbic acid (DHA, the oxidized form of ascorbic acid). Results showed marked decline of ascorbic acid in adrenal glands and kidneys in the F-treated cockerels. Furthermore, the levels of DHA in the kidneys of the F-exposed cockerels increased more than 100% compared with the control levels.[33]

In laboratory mice, both protein and vitamin C were shown to lower F accumulation in bone. For example, mice fed a low-protein diet (containing 4% protein) supplemented with 150 ppm NaF deposited five times more F in the tibia than did control mice fed a regular diet (containing 27% protein) and exposed to the same level of NaF. Furthermore, supplemental vitamin C greatly lowered F deposition in the bone.[34] (It should be noted that mice also produce vitamin C.)

Fluoride treatment has been shown to induce embryotoxicity in pregnant rats.[35] Oral administration of NaF (40 mg/kg body weight) to pregnant rats from day 6 of gestation to day 19 caused decreased body weight, feed consumption, absolute uterine weight, and number of implantations, compared with the control. A higher incidence of skeletal and visceral abnormalities (subcutaneous hemorrhage) was observed in the fetuses of the F-treated pregnant rats. Oral administration of vitamin C (50 mg/kg body weight) with NaF significantly reduced the severity and incidence of F-induced embryotoxicity in the rats.[35]

Exposure of male mice to NaF (10 mg/kg body weight) for 30 days showed a marked decrease in cauda epididymal sperm count, motility, and viability, resulting in significant reduction in fertility rate.[36] Withdrawal of NaF treatment for 30 days produced incomplete recovery. However, when vitamin E or vitamin D was supplemented during the withdrawal period, the toxic effect of NaF was significantly alleviated, as the treated mice restored their reproductive functions and fertility. Additionally, it was found that a combined administration of vitamins D and E was generally more effective than either vitamin D or E administered alone.[36]

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10.10 REVIEW QUESTIONS

1. Why is fluoride the most phytotoxic of the major air pollutants?
2. What are the most important fluoride-containing minerals?
3. List three industrial operations that result in atmospheric emission of fluoride.
4. What is the reason for fluoride emission by the aluminum manufacturing process?
5. What are the characteristic symptoms of leaf injury induced by exposure to high levels of fluoride?
6. How does fluoride affect seed germination?
7. In field studies, what can generally be observed concerning the relationship between bone fluoride level of small animals and the distance of the collection site from a near-by aluminum plant?
8. Explain the dental lesion manifested by animals and humans suffering chronic fluoride poisoning.
9. Of the following fluoride-containing compounds, which one has the highest rate of absorption? Which has the lowest rate of absorption? (a) NaF, (b) cryolite, (c) CaF_2, (d) fluorapatite.
10. What is the suggested mode of action for fluoride?
11. How is fluoride toxicity related to tissue calcium levels?
12. What effect does vitamin C have on fluoride toxicity in animals and humans?
13. How does fluoride inhibit myosin-dependent ATP hydrolysis?
14. How does fluoride affect enolase?
15. How is fluoride related to the protective enzymes?
16. Which nutrients have been shown capable of alleviating fluoride toxicity?

Chapter 11

Volatile Organic Compounds

11.1 INTRODUCTION

Volatile organic compounds (VOCs) are those organic compounds which have a boiling-point within the range 50–100 to 240–260°C.[1] They include a large number of major air pollutants, emitted from both industrial and non-industrial facilities. Chemically, VOCs include aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, some alcohols, esters, and aldehyes. Table 11.1 shows several examples of this group of compounds. The importance of VOCs as a class of air pollutants is evident in view of the fact that the U.S. Environmental Protection Agency (EPA) has designated them as one of the six "Criteria Air Pollutants." In this chapter, the sources, characteristics, and health effects of some examples of VOCs are discussed.

11.2 SOURCES

Both natural and anthropogenic sources contribute to VOC emissions. Natural sources of VOCs include petroleum, forest fires, and the transformation of biogenic precursors. Main anthropogenic sources include high-temperature combustion of fuels, emissions from crude and refined oil, municipal incineration, burning of crops before or after harvesting as an agricultural practice, emissions from powerboats, and others. In terms of the quantities of anthropogenic material emitted from the U.S. during 1998, five major air pollutants, i.e., carbon monoxide (CO), sulfur oxides (SO_x), nitrogen oxides (NO_x), VOCs, and particulate matter (PM), accounted for 98% of total pollution, and VOC emissions alone contributed 14%. Following the passage

Table 11.1 Examples of Volatile Organic Compounds

Group	Examples
Aliphatics	Pentane, hexane, heptane, cyclohexane, octane, nonane, eicosane, dodecane, 2,4-dimethylhexane
Aromatic hydrocarbons	Benzene, diethylbenzene, trimethylbenzenes, dimethyl-ethylbenzene, toluene, xylenes, naphthalene, styrene
Halogenated hydrocarbons	Chloroform, dichlormethane, trichloroethylene, tetrachloroethylene, dichlorobenzenes
Alcohols	2-Butylalcohol, 1-dodecanol
Aldehydes	Decanal, nonanal
Esters	Ethyl acetate, 1-hexyl butanoate

of the Clean Air Act of 1970, VOC emission, like most other emissions, has decreased significantly.

However, a recent survey by the EPA showed indoor pollution as an important source of VOCs. In a survey involving 10 buildings, more than 500 VOCs were detected, though most were detected only once. The study showed that a variety of VOCs originated from common items, such as building materials, cleaning solvents, furnishings, and pesticides. Because indoor concentrations for all compounds except benzene exceeded outdoor levels, it was concluded that indoor sources contributed to the observed results. The importance of indoor VOC pollution in health has been recognized in World Health Organization (WHO) publications.[1]

11.3 PETROLEUM HYDROCARBONS

Petroleum is a complex mixture of hydrocarbons, with a characteristic chemical composition and specific physical properties, depending on the geological and geographical origin of the crude oil and the nature of the cracking process used during refining. Petroleum hydrocarbon components are divided into three major classes, related to their chemical structure: the alkanes, the alkenes, and the aromatics.[2] These compounds enter the environment from both stationary and mobile sources, and comprise a significant portion of the contaminant mixture found in ground and surface waters, coastal areas, and in the global atmosphere.[3,4] The drilling, removal, processing, transportation, storage and use of petroleum hydrocarbons involve several operations, during which losses of material, chemical conversions, and discharges of wastes can occur. Total global emissions and discharges of petroleum have been estimated at about 90×10^6 t.[5]

The specific chemical structures and mixtures of these three classes of petroleum hydrocarbons determine their chemical properties, such as solubility and volatility, and persistence and resistance to photochemical oxidation, microbial degradation, and their biological toxicities in the environment (Table 11.2).

Table 11.2 Comparison Between Aliphatics and Polycyclic Aromatic Hydrocarbons (PAHs)

Characteristics	Aliphatics	PAHs
Rate of degradation	Faster	Slower
Persistence in tissue	Shorter	Longer
Toxicity	Less toxic	More toxic (some are carcinogenic)

11.3.1 Alkanes

11.3.1.1 Properties and Use

The alkanes are chains of carbon atoms with attached hydrogen atoms. They may be simple, straight chains (n-, "normal"), branched (iso-, sec-, tert-), or have a simple ring configuration (cyclo-) (Figure 11.1). Low-molecular-weight alkanes have low boiling points and are highly volatile. They are slightly soluble in water but extremely soluble in fats and oils. The lipophilicity enables rapid penetration through membranes and into tissues. High-molecular-weight alkanes are not soluble in water and are exclusively lipophilic. Low-molecular-weight alkanes are used as solvents, degreasers, and as thinners and diluents of paints, enamels, varnishes and lacquers. They are also used as extractants of organic compounds from plant and animal tissues, soils, and sediments, and in the production of aviation fuels and gasoline.

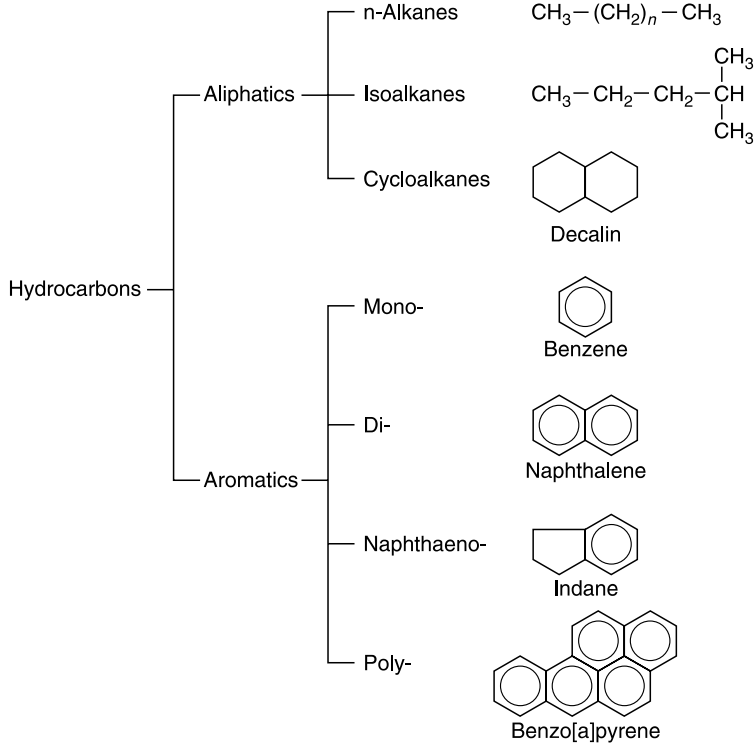

Figure 11.1 Examples of common components of crude petroleum.

11.3.1.2 Health Effects

Alkanes act primarily by solubilizing or emulsifying fats, mucous membranes, and cholesterols. At low concentrations, alkanes are simple irritants and can cause inflammation, redness, itching, and swelling of the skin, mucous membranes, nose, trachea, and bronchioles. They also produce anesthesia and narcosis in the central nervous system (CNS). At high concentrations, acute eczema of the skin and pulmonary edema may develop, as well as unconsciousness or death through asphyxiation caused by paralysis of the portion of the brain responsible for respiration. Alkanes have also been found to penetrate rapidly into the fatty cells of the myelin sheath that surrounds nerve fibers, where they dissolve the cells and cause degeneration of the axons, interrupting the transference of nerve impulses.[6]

Alkanes of higher molecular weight are considered virtually non-toxic, though they may affect chemical communication and interfere with metabolic processes. Many of the same high-molecular-weight alkanes are produced biogenically and have been found to occur naturally in marine organisms.[2]

Alkanes can be excreted, unaltered, by the lungs and can be metabolized by the oxidation of the terminal methyl group by molecular oxygen via the mixed-function oxidase (MFO) system to produce an alcohol. Repeated oxidation of the terminal carbon produces an aldehyde and finally a carboxylic acid, which is broken down by β-oxidation to give rise to acetyl coenzyme A as the final product.[6,7]

In the atmosphere, low-molecular-weight alkanes react with the hydroxyl radical ($OH^{\cdot}$), in a process where a hydrogen atom is abstracted from the alkane to form an alkyl radical. This radical adds molecular oxygen and in the presence of high concentrations of nitric oxide (NO) forms atmospherically reactive nitrogen dioxide (NO_2).[8]

11.3.2 Alkenes

11.3.2.1 Properties and Use

The alkenes are also chains of carbon atoms with attached hydrogen atoms, but the chains contain carbon–carbon double bonds. Alkenes are considered unsaturated in relation to the total possible number of attached hydrogen atoms, compared with an alkane of similar carbon chain length. The double bonds convey a planar configuration to the alkene chains that allows the formation of geometrical isomers (*cis*- and *trans*-). Alkenes are generally more reactive than alkanes, but less reactive than aromatics. They are not found in crude petroleum, but are present in some refined products, specifically gasoline and aviation fuels. Alkenes undergo addition reactions, forming potentially more toxic metabolites. They can undergo polymerization to create long polyethylene chains, oxidation reactions to form oxides that on hydrolysis can form glycols, and halogenation to form extremely toxic chlorinated and brominated hydrocarbon pesticides.

11.3.2.2 Health Effects

In animal experiments, the *cis*- isomers have been found to cause weakness, nausea, and vomiting due to their adverse effects on the gastrointestinal tract, and tremor and cramps due to their effects on the CNS.[6]

11.3.3 Aromatic Hydrocarbons

The aromatic hydrocarbons have a basic structure of six carbon atoms in a ring configuration with six hydrogen atoms and three double bonds, and are unsaturated in terms of attached hydrogen atoms. The aromatic ring may occur in a single, unattached configuration (as in benzene), as two attached rings (to form naphthalene), or as many attached rings. The multi-ring structures are termed polycyclic aromatic hydrocarbons (PAH) (Figure 11.1).

The aromatic ring structures may also include substituted methyl and more complex alkyl side chains, as in the case of toluene, the xylenes, cumene, and 2-methylnaphthalene. The substitution of the hydrogen atoms with other compounds yields several distinct chemical species with varying degrees of polarity, lipophilicity, persistence, and toxicity.[9,10] It is, however, the fundamental ring-structure unit, with the carbon–carbon resonance-stabilized bonds of equal length and energy, that confers great stability to these compounds, making them not only persistent, but some of the most acutely toxic and carcinogenic compounds in the environment.[6,11]

Benzene, toluene, and the three isomers of xylene are among the most common monocyclic aromatic compounds found in petroleum.[12] Compared with other alkanes, alkenes, and polycyclic aromatic hydrocarbons, they have low molecular weight, low water-solubility, high volatility and flammability, and have the same toxicological modes of action – narcosis.[10,13] Their structures, stability, and ability to be both slightly hydrophilic and lipophilic enhance their accessibility to many niches, species, biochemical pathways and sites of action. This accounts, in part, for the designation of these compounds as priority pollutants by the EPA.[10,12]

11.3.3.1 Benzene

Benzene (boiling point 80.1°C) is chemically the most significant aromatic hydrocarbon because it is the starting material for the manufacture of numerous industrial and agricultural products.[6,10,12] It has been in commercial use for over a century and its toxic effects have been suspected for almost as long.[10] Benzene has a wide variety of uses in industrial society. Some examples include:

- an intermediate in the synthesis of pharmaceuticals and other chemicals, such as styrene, detergents, pesticides, and cyclohexane
- a degreasing and cleaning agent
- an antiknock fuel additive[14]

- a solvent for extracting pesticides from tissues, soils, and sediments in research and industrial applications
- a thinner and diluent of paints, inks and lacquers
- as a solvent in the rubber industry[12]

In the atmosphere, benzene and more than 70 of its derivatives are present as a result of combustion of fossil fuels and emissions from a variety of industrial processes. The principal chain reactor is the $OH^{\cdot}$ radical, which can either add to the aromatic ring or abstract a hydrogen atom from the side group. A variety of aromatic aldehydes, alcohols, and nitrates are produced in this way, as well as products of ring cleavage. These products have moderately high molecular weight and are moderate solubility in water; therefore, they can be readily deposited on aerosol particle surfaces.[8]

The toxicological mode of action of benzene is narcosis, affecting the CNS. At high concentrations, inhalation of air containing approximately 64 g/m^3 of benzene can be fatal within a few minutes, and one tenth of that level can cause acute poisoning within an hour.[6] Exposure causes skin irritation, fluid accumulation in the lungs (edema), excitation, depression, and may eventually lead to respiratory failure and death. At lower concentrations, benzene can cause blood abnormalities, lower white-cell count and bone-marrow damage.[6,15] These toxicological effects have been attributed specifically to the *trans*-benzene-1,2-oxide intermediate formed during eucaryotic oxidative degradation of benzene.[6] During oxidation, the oxygen atom is incorporated directly into the ring, forming an epoxide intermediate (Figure 11.2). The epoxide, which is not immediately degraded, resides in the cell structures and actively reacts with cell nucleophiles, damaging blood, lymph, and bone-marrow cells, as well as affecting liver and kidney function.[10] The epoxide is eventually converted to phenol by a slower, nonenzymatic, rearrangement process, and the phenol is finally eliminated from the body as its sulfate.[6,9]

Benzene is of most concern because it is known to be associated with the development of leukemic cancer in humans.[16] Although the mechanisms by which benzene-induced toxicity and induction of leukemia are not yet fully understood, they are known to be complicated by various pathways, including those of metabolism, oxidative stress, DNA damage, cell-cycle and growth regulation, and programmed cell death (apoptosis). A recent report by Yoon et al.[17] has enhanced the knowledge of the mechanisms involved in benzene toxicity. By using mouse bone-marrow tissue in their studies, the researchers concluded that the observed effects of benzene on bone-marrow cells results from:

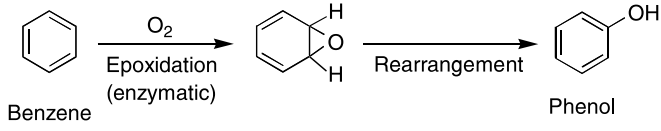

Figure 11.2 Biotransformation of benzene.

- cellular damage due to benzene metabolites and oxidative stress
- dysfunction of the machinery of cell cycle arrest for repairing damaged DNA, resulting in continuous cycling of damaged cells even without undergoing repair
- inhibition of apoptosis by both disruption of p53-dependent proapoptotic signaling and activation of survival genes
- failure of activating DNA repair genes, which may lead to the increase in cell mutation frequencies at the candidate DNA locus responsible for benzene carcinogenesis, resulting in the development of hemopoietic malignancies.

11.3.3.2 Toluene

Toluene (boiling point 110.6°C) is produced primarily as a precursor for the synthesis of other chemicals. For example, 70% of the product is used for the synthesis of benzene, 15% for the manufacture of other chemicals, and 10% is used as a solvent for paints and as a gasoline additive.[12,13] Toluene is used as one of the major substitutes for benzene because of the extreme hazards associated with benzene exposure.[6]

The toxicological mode of action for toluene is narcosis. At low concentrations it produces skin irritations and at higher levels it affects blood cells, the liver, kidney and the CNS (through which it causes headaches, nausea, and impaired coordination).[6] Compared with benzene, toluene is less water-soluble and more lipophilic, causing greater concentrations of it to be more rapidly transported to the site of action and so increasing its potential for toxic effects.[18] However, while toluene's methyl group increases concentration and depressant effects at the site of action, the rapid enzymatic degradation of toluene immediately reduces the concentration, limiting the potential toxicological effects and resulting in a lowered observed toxicity.[3,18,19] The mechanism involved in moderating the toxic effects is the rapid oxidation of the aliphatic methyl side-chain rather than the ring structure. Benzyl alcohol and benzoate intermediates are formed, which are conjugated to hippuric acid (about 70% of the dose is affected) and rapidly eliminated, with the remainder being exhaled from the lungs unchanged.[6,9]

11.3.3.3 Xylenes

The xylenes (ortho [*o*-], meta [*m*-] and para [*p*-] – boiling points 144.4°C, 139.1°C, and 138.3°C, respectively), have also been used as replacements for benzene and toluene in the production of resins, synthetic fabrics, plastics, and as gasoline additives, cleaners, solvents, and lacquers.[6,11,13] As in the case of toluene and benzene, the xylenes act as narcotics on the CNS, causing headaches, impaired coordination, edema, and nausea at higher concentrations; and skin irritations, anemia, blood-cell damage, and a decrease in blood platelets at lower, chronic-exposure levels.[6] In oxidative degradation, *m*-xylene and *p*-xylene are metabolized to *m*-toluates and *p*-toluates, which are further

oxidized by the meta pathway.[6,20,21,22] *o*-Xylene oxidation does occur, but by a modified co-metabolic pathway with toluene.[20] Elimination of xylenes is primarily through the excretion of metabolites in the form of methyl hippuric acid (95% of the absorbed dose), 1 to 2% as xylenol, and by exhalation of 3 to 5% as the unchanged solvent.

The double methylation of the xylenes makes them virtually insoluble in water. They are very lipophilic, with the potential for rapid transport to the site of action. As with toluene, the toxicity of the xylenes is mediated by the reduction in their water-soluble fraction concentrations and their rapid biodegradation. The presence of the second methyl group on the benzene ring determines the number of enzymatic steps in the xylene degradation process and the specific pathway, rate of degradation, and potential for bioaccumulation by its location at the ortho, meta, or para position.[3,18,19,20,21,22]

11.4 POLYCYCLIC AROMATIC HYDROCARBONS

11.4.1 Introduction

Polycyclic or polynuclear aromatic hydrocarbons (PAHs) are a group of compounds composed of two or more fused aromatic rings in linear, angular, or cluster arrangements. By definition, they consist solely of carbon and hydrogen.[23]

The EPA has focused on the 16 PAHs that are included on their list of 126 priority pollutants (Table 11.3). These were selected on the basis of toxicity, potential for human exposure, and frequency of occurrence at hazardous waste sites.

The reason for the concern over PAHs is that many have been shown to be carcinogenic to animals and substantial data exist incriminating them as carcinogenic to humans.[24] Eight of the PAHs in Table 11.3 are classified as Group B2 compounds, probable human carcinogens. The remaining eight are classified as Group D compounds, which means that insufficient data are available to assess their carcinogenic potential. No individual PAH has been classified as belonging to Group A, known human carcinogens. However, several complex mixtures from which PAHs have been identified are known human carcinogens, such as cigarette smoke, coal tar, and coke-oven emissions.

11.4.2 Sources

PAHs are ubiquitous and can occur in the air attached to dust particles, or in soil and sediments as solids. PAHs have also been detected in food and water.[24] The predominant source of PAHs is the incomplete combustion of organic material. Anthropogenic sources of PAHs can be divided into stationary and mobile categories of emission.[23] Vehicular engines are the major contributors

Table 11.3 Polycyclic Aromatic Hydrocarbons Identified as Priority Pollutants by the U.S. Environmental Protection Agency

Name	Abbreviation	Carcinogenic classification
Acenaphthylene	Ace	D[a]
Acenaphthene	–	D
Anthracene	Ant	D
Benz(a)anthrecene	BaA	B2[b]
Benzo(a)pyrene	BaP	B2
Benzo(b)fluoranthene	BbF	B2
Benzo(k)fluoranthene	BbK	B2
Benzo(g,h,i)perylene	Bpe	B2
Chrysene	Chr	B2
Dibenz(a,h)anthracene	DbA	D
Fluoranthene	Fth	B2
Fluorene	Fl	D
Ideno(1,2,3-c,d)pyrene	IP	B2
Naphthalene	Na	D
Phenanthrene	Phe	D
Pyrene	Pyr	D

[a]B2: probable human carcinogens.
[b]D: insufficient data are available to assess their carcinogenic potential.

to the mobile emissions. The stationary fraction encompasses a wide variety of combustion processes, including residential heating, aluminum production, coke manufacture, incineration,[25] and power generation. The amounts and types of PAH produced by each of these vary widely due to differences in fuel type and combustion conditions.[23]

Not all PAHs are the result of human activity. Volcanic eruptions and forest and prairie fires are among the major sources of naturally produced PAHs. In addition, there is some evidence that PAHs may also be formed by direct biosynthesis by microbes and plants.[26]

11.4.3 Physical and Chemical Properties

As pure chemicals, PAHs generally exist as colorless, white, or pale yellow-green solids. However, the physical and chemical characteristics of PAHs vary according to their molecular weight.[26] Resistance to oxidation and reduction tends to decrease with increasing molecular weight. Vapor pressure and aqueous solubility decrease almost logarithmically with increasing molecular weight. As a consequence of these differences, they tend to be environmentally more stable because they are less amenable to microbial degradation.[27]

11.4.4 Transport

PAHs released to the atmosphere are subjected to short- and long-range transit and are removed via wet and dry deposition. In surface water, PAHs can

volatilize, photodegrade, oxidize, biodegrade, bind to particulates or accumulate in organisms. In sediments, PAHs can biodegrade or accumulate in aquatic organisms. PAHs in soils can biodegrade or accumulate in plants, or may enter the groundwater and be transported within an aquifer. Figure 11.3 illustrates major routes involved in the transport of PAHs.

11.4.5 Exposure

PAHs are widely distributed in the environment, and have been detected in air, water, sediment, soil, food, and consumer products such as cosmetics and cigarettes. As a result, humans are exposed to these chemicals as part of everyday living. As stated previously, most of the direct releases of PAHs into the environment are to the atmosphere. Important sources of PAHs in surface waters include deposition of airborne PAHs, municipal wastewater discharge, urban storm-water runoff, and industrial discharges.[27] Most of the PAHs in surface waters and soils are believed to result from atmospheric deposition.

Food groups that tend to have the highest levels of PAHs include charcoal-broiled or smoked meats, leafy vegetables, grains, and vegetable fats and oils.[28] The presence of PAHs on leafy vegetables and grains is believed to be caused by atmospheric deposition and reflects local conditions in the growing area. The average American is estimated to consume between 1 and 5 μg/day of carcinogenic PAHs, with unprocessed grains and cooked meats as the greatest

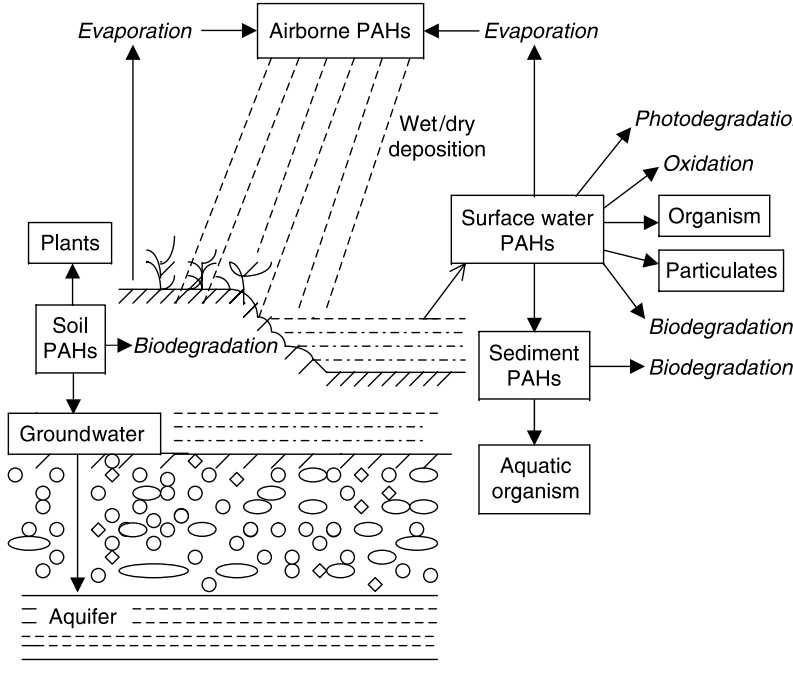

Figure 11.3 Transport of polycyclic aromatic hydrocarbons (PAHs).
Source: Yu, M.-H., personal communication.

source of these substances (carcinogenic being defined as group B2 compounds).[28] A person who consumes a heavy meat diet has the highest estimated potential dose, on the order of 6 to 12 μg/day. A vegetarian diet can offer an elevated PAH intake, 3 to 9 μg/day, if the diet comprises leafy vegetables, such as lettuce and spinach, and unrefined grains.

Using the EPA's assumption of an average respiration rate of 20 m^3/day, the estimated potential PAH dose by inhalation by nonsmokers ranges between 0.02 and 3 μg/day, with a median value of 0.16 μg/day.[28] Tobacco smoke can be a major source of airborne carcinogenic PAHs. Mainstream smoke from unfiltered cigarettes may contain 0.1 to 0.25 μg/cigarette. An individual who smokes one pack of unfiltered cigarettes a day is estimated to inhale an additional 2 to 5 μg/day. Indoor air levels associated with tobacco smoke have been reported in the range of 3 to 29 ng/m^3.[28] The consequence is that exposure to secondary cigarette smoke may be implicated in adverse health effects.

The potential dose of carcinogenic PAHs from drinking water (assuming an average drinking water consumption of 2 l/day), ranges between 0.2 and 120 ng/day, with a median value of 6 ng/day.[27] Drinking water concentrations have been reported to range between 0.1 and 61.6 ng/l, with most drinking water values falling between 1 and 10 ng/l.

Carcinogenic PAHs are found in all surface soils,[28] with urban areas having higher concentrations than do agricultural and forest soils. Typical concentrations of carcinogenic PAHs are in the range of 5 to 100 μg/kg: agricultural soils contain 10 to 100 μg/kg, and urban soils contain 0.6 to 3 mg/kg. Assuming rate of incidental ingestion of soil of 50 mg/day, the potential intake of carcinogenic PAHs for urban populations ranges from 0.003 to 0.4 μg/day, with the median value being 0.06 μg/day.

Excluding occupational exposure routes, food may be the major source of carcinogenic PAHs for nonsmokers. Smokers of nonfiltered cigarettes may be exposed to twice the concentration of carcinogenic PAHs.

11.4.6 Metabolism

PAHs enter the human body quickly by all routes of exposure: inhalation, ingestion, and dermal contact.[24] The rate of absorption is increased when the PAHs are present in oily mixtures. PAHs are conveyed to all the tissues of the body containing fat, and tend to be stored mostly in the kidney, liver, with smaller amounts in the spleen, adrenal glands, and ovaries. Results from animal studies show that PAHs tend not to be stored in the body for prolonged periods, and are usually excreted within a matter of days.

The lipophilicity of PAHs enables them to readily penetrate cellular membranes. Subsequent metabolism renders them more water-soluble and thus more readily removed from the body. However, PAHs can also be converted to more-toxic or carcinogenic metabolites. One factor that may influence the delicate balance of toxification and detoxification is the site at which the chemically reactive metabolite is formed. Metabolism of PAHs occurs in all tissues.[26] The extent of induction of enzyme systems following exposure to

xenobiotics is known to vary with tissues. For example, the liver is generally more inducible than the lung or skin.

In mammals, the cytochrome P450 MFO system is responsible for initiating the metabolism of xenobiotics. As discussed in Chapter 6, the primary function of this system is to render lipophilic compounds more water-soluble. Although this system effectively detoxifies certain xenobiotics, others, such as PAHs, are transformed into intermediates that are highly toxic, mutagenic, or carcinogenic to the host. For example, oxidative metabolism of benzo[a]pyrene by the MFO system converts it into a dihydroxy epoxide, believed to be a carcinogen that can interact with DNA (see Chapter 16).

The PAHs are very resistant to degradation due to their complex ring structures. As a result, these compounds have the potential to recycle and participate in atmospheric reactions several times before being degraded enough to be removed from the environment. The same resistance and persistence of these chemicals occur in terrestrial and aquatic systems, making these compounds the most hazardous in terms of long-term, chronic exposure to their carcinogenic and mutagenic properties. Furthermore, recent studies show that the toxicity of PAHs increases following photomodification by natural sunlight.[29]

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11.6 REVIEW QUESTIONS

1. What are VOCs? What are the major groups of VOCs?
2. List some of the major anthropogenic sources of VOCs.
3. Chemically, what are the three major classes of petroleum?
4. What is narcosis? What types of VOCs can cause it?
5. Why are aromatic hydrocarbons designated priority pollutants by the EPA?
6. What is the effect of low concentrations of benzene?
7. How is benzene metabolized in the body? In what form is it excreted?
8. Name three environmentally important monoaromatic compounds.
9. What is the concern over PAHs?
10. Briefly explain the fate of the PAHs in surface water.
11. What foods contain relatively high levels of PAHs?
12. Explain the process involved in the metabolism of benzo[a]pyrene and indicate the consequence of the metabolism.

Chapter 12

Soil and Water Pollution – Environmental Metals and Metalloids

12.1 INTRODUCTION

The metals found in the environment are derived from a variety of sources. Such sources include: natural weathering of the earth's crust, mining, soil erosion, industrial discharge, urban runoff, sewage effluents, pest or disease control agents applied to plants, air pollution fallout, and a number of others.[1] Since the Industrial Revolution, the use of metals has been a mainstay of the economy in many developed countries, particularly the U.S. However, the increase of mining for metal ores, as well as the combustion of coal as an important energy source in many countries, has led to the health and exposure risks to workers and the public becoming of increasing concern.

While some metals found in the environment are essential nutritionally, others are not. The latter include some *heavy metals*, a group of metallic elements that exhibit certain chemical and electrical properties. Heavy metals generally have a density greater than $5\,g/cm^3$,[2] and an atomic mass exceeding that of calcium. Most of the heavy metals are extremely toxic because, as ions or in certain compounds, they are soluble in water and can be readily absorbed into plant or animal tissue. After absorption, these metals tend to bind to biomolecules such as proteins and nucleic acids, impairing their functions.

For a long time, the effects of toxic heavy metals on living organism were considered almost exclusively a problem of industrial exposure and of accidental childhood poisonings. Until recently, much of the literature concerning the subject dealt with experiments relating to exposure of children to lead-based paint. Although significant progress has been made in reducing the levels of a number of toxic metals in the environment, as exemplified by the marked reduction in atmospheric lead (Pb) pollution in the past three decades, problems with heavy metals still exist in many parts of the world. According to the U.S. Centers for Disease Control and Prevention (CDC), Pb poisoning is the most common and serious environmental disease affecting young children.

This chapter examines the sources of several metals and a metalloid, and their health and biological effects on living organisms. The discussion includes Pb, cadmium (Cd), mercury (Hg), nickel (Ni), and arsenic (As). These and a number of other metals are widely used in industry, and Pb, Cd, and Hg, in particular, are generally considered the most toxic to humans and animals.

12.2 LEAD

12.2.1 Characteristics and Use of Lead

Lead occurs naturally, in small amounts, in the air, surface waters, soil, and rocks. Because of its unique properties, Pb has been used for thousands of years. Its high ductility (the quality of being ductile, i.e., capable of being permanently drawn out without breaking) and malleability have made Pb the choice material for a large number of products, including glass, paint, pipes, building materials, art sculptures, print typeface, weapons, and even money. The use of Pb has accelerated since the Industrial Revolution, and particularly since World War II. However, its wide use has resulted in elevated Pb concentrations in the ecosystem. For example, in locations where Pb is mined, smelted, and refined, where industries use the metal, and in urban–suburban complexes, the environmental Pb levels are greatly increased. Until recently, the primary source of environmental Pb in many countries was the combustion of leaded gasoline.

Lead has the low melting point of 327°C. It is extremely stable in compound forms, therefore dangerous forms may remain in the environment for a long time. This stability made it the first choice for high-quality paint because it resisted cracking and peeling and retained color well. Millions of tons of lead-based paint were used in the U.S. before it was banned in 1978. (Europe banned the use of Pb paint in residences in 1921.) Because Pb is ubiquitous and is toxic to humans at high doses, levels of exposure encountered by some population groups constitute a serious public health problem.[3] The importance of Pb as an environmental pollutant is indicated by the fact that the U.S. Environmental Protection Agency (EPA) has designated the metal as one of the six "Criteria Air Pollutants."

12.2.2 Sources of Lead Exposure

12.2.2.1 Airborne Lead

Airborne Pb pollution is a growing problem facing many countries. Early Pb poisoning outbreaks were associated with the burning of battery shell casings. Industrial emissions of Pb also became a concern as the Industrial Revolution progressed. Increasing Pb pollution in the environment was first revealed in a 1954 study conducted by a group of scientists from the U.S. and Japan on the Pb contents of an arctic snow pack in Greenland. In the study, the scientists found steady increases in Pb levels, beginning around the year 1750. Sharp increases were evident after the end of World War II. Importantly, the content of other minerals in the snow pack was found to remain steady. These observations suggest that increasing atmospheric Pb pollution is a consequence of human activities.[4]

The main industrial sources of Pb pollution include smelters, refineries, incinerators, power plants, and manufacturing and recycling operations. For

example, Kellogg, a small town in Idaho, lies in a deep valley directly downwind of the Bunker Hill lead smelter. Beginning in 1974, about 200 children between the ages of 1 and 9 years were screened annually for blood Pb levels. Until the closure of the plant in 1983, after 100 years of operation, Kellogg children's blood Pb levels were among the highest in the U.S. Since the plant closed, screenings showed a steady decrease in children's blood Pb levels. In 1986, the average level was about the same as in children who had not lived near a smelter, with most levels falling below the established action level of 25 μg/dl.[5]

Until recently, the number one contributing factor of Pb air pollution was, however, the automobile. The introduction of tetraethyl lead as an antiknock agent in gasoline in the 1920s resulted in a steep increase in Pb emission. During combustion, Pb alkyls decompose into lead oxides and these react with halogen scavengers (used as additives in gasoline), forming lead halides. Ultimately, these compounds decompose to lead carbonate and oxides. However, a certain amount of organic Pb is emitted from the exhaust. It was estimated earlier that about 90% of the atmospheric Pb was due to automobile exhaust and that worldwide a total of about 400 t of particulate Pb was emitted daily into the atmosphere from gasoline combustion. Since the mandatory use of unleaded gasoline in the U.S. began in 1978, followed by improved industrial-emission control, atmospheric Pb emission from major sources in the U.S. has decreased dramatically. According to the EPA, annual Pb emission from major emission sources in the U.S. decreased from 56,000 t in 1981 to 7100 t in 1990.[6] While atmospheric Pb pollution has also decreased in other developed countries, a similar trend has not occurred in many developing countries. This is particularly true in several less-developed countries that are experiencing rapid economic development.

12.2.2.2 Waterborne Lead

Although Pb emissions into the environment have declined markedly as a result of the decreased use of leaded gasoline, Pb is still a potential problem in aquatic systems because of its industrial importance. Once emitted into the atmosphere or soil, Pb can find its way into the aquatic systems. Both surface water and groundwater may contain significant amounts of Pb derived from these sources.

Water is the second largest source of Pb for children (Pb in paint being the largest). In 1992, the levels of Pb in 130 of the 660 largest municipal water systems in the U.S., serving about 32 million people, were found to exceed the action level of 15 ppb set by the EPA. Many homes are served by Pb service lines or have interior pipes of Pb or copper (Cu) with Pb solder.[7]

Another serious problem related to waterborne Pb is from lead shot left in lakes and ponds. Although non-lead shot is now in use, much lead shot remains in aquatic systems. A large number of waterfowl in the U.S. are poisoned or killed annually as a result of ingesting lead shots. For example, according to a bird-rehabilitation center in Whatcom County, Washington,

lead shot killed nearly 1000 swans in the county and adjacent areas in British Columbia, Canada, in the five years following the center's opening. The investigators at the center indicated they were unable to pinpoint the source of the lead shot that had killed the birds.

12.2.2.3 Lead in Food

Food is a major source of Pb intake for humans and animals. Plant food may be contaminated with Pb through its uptake from ambient air and soil, animals may then ingest the Pb-contaminated vegetation. In humans, Pb ingestion may arise from eating Pb-contaminated vegetation or animal foods. Vegetation growing near highways has long been known to accumulate high quantities of Pb from automobile exhaust.[8] However, recent studies show that in the U.S. the levels of Pb in such vegetation have decreased significantly following the general use of unleaded gasoline. Another source of ingestion is through the use of Pb-containing vessels or Pb-based pottery glazes.

About 27 million housing units were built in the U.S. before 1940, when Pb was in common use, and many old houses still exist.[9] The eventual deterioration of these houses continues to cause exposure of children to Pb. Young children eat flaking paint from the walls of these houses – a phenomenon called *pica*. The risk of this practice to children has been widely recognized.

12.2.2.4 Lead in Soils

Almost all of the Pb in soil comes from Pb-based paint chips from homes, factory pollution, and the use of leaded gasoline. In the U.S., emission of Pb through various uses of the metal is estimated at 600,000 t/year. Countless additional tonnes are dispersed through mining, smelting, manufacturing, and recycling. Disposal of Pb-based paint is a further cause of soil contamination, as is use of Pb in insecticides. Earlier studies showed that about 50% of the Pb emitted from motor vehicles in the U.S. was deposited within 30 m of the roadways, with the remainder scattered over large areas.[10] Lead tends to stick to organic matter in soils; most of the Pb is retained in the top several centimeters of soil, where it can remain for years. Soil contamination also leads to other problems associated with Pb-contaminated foods.

12.2.3 Lead Toxicity

12.2.3.1 Lead Toxicity to Plants

Plants can absorb and accumulate Pb directly from ambient air and soils. Lead toxicity to plants varies with plant species and the other trace metals present. For example, barley plants are very sensitive to Pb.[11] Lead has been shown to inhibit seed germination by suppressing general growth and root elongation.[12,13] The inhibitory effect of Pb on germination, however, is not as severe

as that exhibited by several other metals. For example, in a study on the effect of Cr, Cd, Hg, Pb, and As on the germination of mustard seeds (*Sinapis alba*), Fargasova[1] showed that after 72 hours the most toxic metal for seed germination was As^{5+}, while the least toxic was Pb^{2+}. According to Koeppe,[12] Pb might be bound to the outer surface of plant roots, as crystalline or amorphous deposits, and could also be sequestrated in the cell walls or deposited in vesicles. This might explain the higher concentrations of Pb in roots[14] and can explain the low toxic effect on mustard seeds. Pb may be transported in plants following uptake, and can decrease cell division, even at very low concentrations. Koeppe and Miller[15] showed that Pb inhibited electron transport in corn mitochondria, especially when phosphate was present.

12.2.3.2 Lead Poisoning in Animals and Fish

Young animals have been shown to be more susceptible to Pb poisoning than are adults. For example, growing rats accumulated more Pb in their bones than did adult rats, and one-week-old suckling rats absorbed Pb from their intestinal tract much more readily than adults.[16,17]

In aquatic systems, acidification of waters is an important factor in Pb toxicity. Eggs and larvae of common carp (*Cyprinus carpio*) exposed to Pb at pH 7.5 showed no significant differences in mortality compared with the control. At pH 5.6, there was no significant mortality in the Pb-exposed eggs, but the larvae showed significant mortality at all treatment levels. Additionally, a marked change in the swimming behavior was observed with the exposed larvae; the majority were seen lying at the bottom of the test chamber, in contrast to the free-swimming controls. Pb exposure also influenced heartbeat and tail movements; heart rate increased and tail movements decreased with increasing Pb concentrations. Subsequent studies showed that Pb uptake and accumulation increased with decreasing pH values.[18] The influence of Pb on freshwater fish also varies, depending on species exposed. For instance, goldfish are relatively resistant to Pb, which may be due to their profuse gill secretion.

As mentioned previously, ingestion of Pb shot from lakes and fields has resulted in the death of a large number of birds in the U.S. Lead ingested by a bird paralyzes the gizzard; death follows as a result of starvation.

12.2.3.3 Health Effects of Lead in Humans

In humans, about 20 to 50% of inhaled, and 5 to 15% of ingested inorganic Pb is absorbed. In contrast, about 80% of inhaled organic Pb is absorbed, and ingested organic Pb is absorbed readily. Pb ingestion in the U.S. is estimated to range from 20 to 400 μg/day. An adult absorbs about 10% of ingested Pb, whereas for children the value may be as high as 50%. Once in the bloodstream, Pb is primarily distributed among blood, soft tissue, and mineralizing tissue (Figure 12.1). The bones and teeth of adults contain more

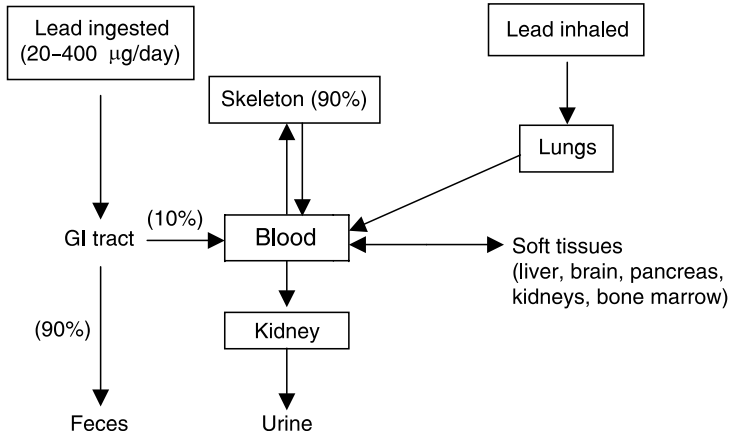

Figure 12.1 Metabolism of lead in humans.

than 95% of the total body burden of Pb. In times of stress, the body can metabolize Pb stores, thereby increasing its levels in the bloodstream. Lead is accumulated over a lifetime and released very slowly. In single exposure studies with adults, Pb has a half-life in blood of approximately 25 days. In soft tissue the half-life is about 40 days, and in the non-labile portion of bone it is more than 25 years.

Lead toxicity has been known for over two thousand years. The early Greeks used Pb as a glazing for ceramic pottery and became aware of its harmful effects when it was used in the presence of acidic foods. Researchers suggest that some Roman emperors became ill, and even died, as a result of Pb poisoning from drinking wines contaminated with high levels of Pb.

Lead is found in all human tissues and organs, though it is not needed nutritionally. It is known as one of the *systemic poisons* because, once absorbed into the circulation, Pb is distributed throughout the body, where it affects various organs and tissues. It inhibits hematopoiesis (formation of blood or blood cells) because it interferes with heme synthesis (see below), and Pb poisoning may cause anemia. Pb also affects the kidneys by inducing renal tubular dysfunction. This, in turn, may lead to secondary effects. Effects of Pb on the gastrointestinal tract include nausea, anorexia, and severe abdominal cramps (lead colic) associated with constipation. Pb poisoning is also manifested by muscle aches and joint pain, lung damage, difficulty in breathing, and diseases such as asthma, bronchitis, and pneumonia. Pb poisoning can also damage the immune system, interfering with cell maturation and skeletal growth. Pb can pass the placental barrier and may reach the fetus, causing miscarriage, abortions and stillbirths.

According to the CDC, lead poisoning is the most common and serious environmental disease affecting young children.[19] Children are much more vulnerable to Pb exposure than adults because of their more rapid growth rate and metabolism. Pb absorption from the gastrointestinal tract in children is

also higher than in adults (25% vs. 8%), and ingested Pb is distributed to a smaller tissue mass. Children also tend to play and breathe closer to the ground, where Pb dust concentrates. One particular problem has been the Pb poisoning of children who ingest flakes of lead-based paint. This type of exposure accounts for as much as 90% of childhood Pb poisoning. The main health concern in children is retardation and brain damage. High exposure may be fatal.

The developing fetus is also highly susceptible to Pb. According to the Public Health Service, in 1984 more than 400,000 fetuses were exposed to Pb through maternal blood. Pb is associated with early developmental effects, and the developing nervous system in children can be adversely affected at blood Pb levels of less than 10 μg/dl.

The primary target organ for Pb is the central nervous system (CNS). Lead can cause permanent damage to the brain and nervous system, resulting in such problems as retardation and behavioral changes. Of greatest current concern is the impairment of cognitive and behavioral development in infants and young children. Because of this, CDC lowered the definition of elevated blood Pb level for children under the age of 6 years from 25 to 10 μg Pb/dl.[19] The median Pb levels in children under the age of 6 years decreased from about 15 to 18 μg/dl blood in 1970 to 2 to 3 μg Pb/dl in 1994 as a result of the concurrent reduction of Pb in automotive emissions, paint, drinking water, and soldered food cans. However, more than 2.2% of children ages 1 to 5 years still have blood Pb concentrations above 10 μg/dl. Statistics also show that 17% of children in the U.S. are at risk of Pb poisoning.

According to the International Agency for Research on Cancer (IARC), lead acetate ($[CH_3COO]_2Pb$) and lead phosphate ($Pb_3[PO_4]_2$) are designated as "reasonably anticipated to be human carcinogen," based on sufficient evidence of carcinogenicity in animal experiments. When administered in the diet of rats, lead acetate induced renal adenomas and carcinomas and cerebral gliomas. Subcutaneous injections of lead phosphate induced renal cortical tumors. However, there is inadequate evidence for determining the carcinogenicity of lead acetate and lead phosphate in humans.[20]

12.2.4 Biological Effects of Lead

In plants, Pb has been shown to inhibit electron transport in corn mitochondria,[15] depress respiratory rate in germinating seeds, and inhibit various enzyme systems.

As a systemic poison, Pb can cause many adverse effects in different tissues. It may be expected that these abnormalities are somehow related to biochemical changes. Although the mechanisms involved in Pb toxicity are complex, several examples are given below.

As an electropositive metal, Pb has a high affinity for the sulfhydryl (–SH) group. As discussed in Chapter 4, an enzyme that depends on the –SH group as the active site will be inhibited by Pb. In this example, Pb reacts with the –SH group on the enzyme molecule to form mercaptide, leading to inactivation.

Reaction 12.1 shows the chemical reaction between the Pb^{2+} ion and two –SH-containing molecules:

$$2RSH + Pb^{2+} \rightarrow R–S–Pb–S–R + 2H^{+} \quad (12.1)$$

Examples of the SH-dependent enzymes include adenyl cyclase and aminotransferase. Adenyl cyclase catalyzes the conversion of ATP to cyclic AMP (cAMP) needed in brain neurotransmission. Aminotransferase is involved in transamination and thus is important in amino acid, and therefore protein, metabolism.

Because the divalent Pb^{2+} ion is similar in many ways to the Ca^{2+} ion, Pb may exert a competitive action in processes such as mitochondrial respiration and neurological functions. In mammals, Pb can compete with calcium (Ca) for entry at the presynaptic receptor. Because Ca evokes the release of acetylcholine (ACh) across the synapse (see Chapter 13), this inhibition manifests itself in the form of decreased end-plate potential. The miniature end-plate potential release of subthreshold levels of ACh is shown to be increased.[21] The chemical similarity between Pb and Ca may partially account for the fact that they seem interchangeable in biological systems, and that 90% or more of the total body burden of Pb is found in the skeleton.

Lead causes adverse effects on nucleic acids, leading to either decreased or increased protein synthesis. Pb has been shown to decrease amino acid acceptance by tRNA, as well as the ability of tRNA to bind to ribosomes. Pb also causes disassociation of ribosomes. The effects of Pb on nucleic acids, therefore, have important biological implications.[21]

One of the most widely known biochemical effects of Pb is the inhibition of δ-aminolevulinic acid dehydratase (ALA-D)[22] and ferrochelatase,[23] two key enzymes involved in heme biosynthesis. ALA-D is responsible for the conversion of δ-aminolevulinic acid into porphobilinogen, whereas ferrochelatase catalyzes the incorporation of Fe^{2+} into protoporphyrin IX to form heme (Figure 12.2). Inhibition of these two enzymes by Pb therefore severely impairs heme synthesis. ALA-D inhibition by Pb is readily exhibited because the enzyme activity is closely correlated with blood Pb levels. An increased excretion of δ-aminolevulinic acid in urine provides evidence of increased Pb exposure. A concomitant decrease in blood porphobilinogen concentrations also occurs. These observations have been utilized in experimental and clinical laboratory studies involving Pb poisoning.

Lead inhibition of ALA-D is likely due to the interaction of Pb with zinc (Zn), which is required for the enzyme. Alternatively, the mode of action of Pb in ferrochelatase inhibition may be related to its competition with iron (Fe) for binding sites on proteins.

12.2.5 Lead and Nutrition

Nutritional factors can influence the toxicity of Pb in humans by altering its absorption, metabolism, or excretion. Several nutrients affect the absorption of

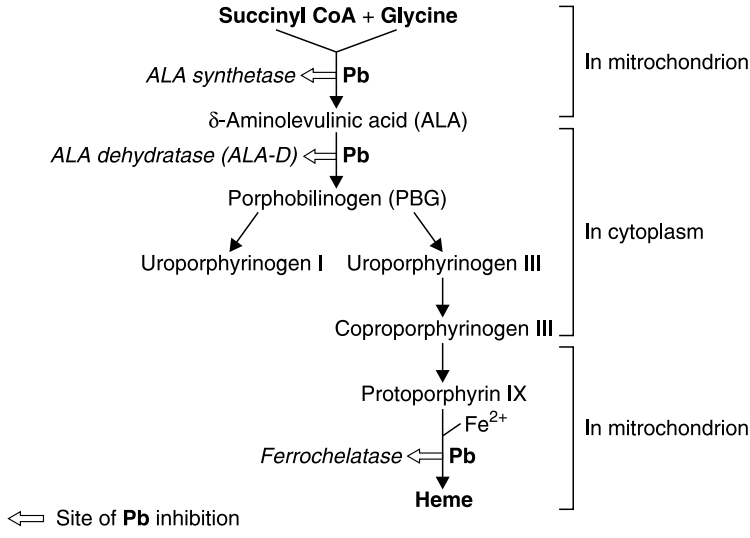

Figure 12.2 Lead inhibition of heme synthesis.

Pb from the gastrointestinal tract. These include Ca, phosphorus (P), Fe, lactose, fat, and vitamins C, D, and E. Low intakes of Ca, P, and Fe, for example, may increase Pb absorption[20] or decrease Pb excretion, resulting in higher toxicity, while a high fat intake may lead to increased Pb accumulation in several body tissues.

Calcium, P, and Fe have been shown to reduce Pb absorption. Competition for mucosal binding proteins is one mechanism by which Ca reduces the intestinal absorption of Pb. The absorption of Pb is increased in Fe-deficient animals, therefore Fe-deficiency may contribute to the incidence of Pb poisoning in exposed persons. Other nutrients, such as Zn and magnesium (Mg) also affect the metabolism of Pb, especially the placental transfer of Pb from pregnant mother to fetus.[24,25]

The effect of vitamin C on Pb toxicity appears to be complex. Whereas both vitamins C and D increase Pb absorption, vitamin C may also lower Pb toxicity. Vitamin E also affects Pb toxicity. In the blood, Pb can react directly with the red blood cell membrane, causing it to become fragile and more susceptible to hemolysis. This may result in anemia. Splenomegaly (enlargement of the spleen) occurs when the less flexible red blood cells become trapped in the spleen. It is suggested that Pb may mark the red blood cells as abnormal and contribute to splenic destruction of the cells. Pb may act as an oxidant, causing increased lipid peroxidation damage. Vitamin E is an antioxidant and can therefore limit peroxidation process and damage. Less severe anemia and splenomegaly are observed in Pb-poisoned rats fed diets containing supplemental vitamin E.

12.3 CADMIUM

12.3.1 Introduction

The outbreak of itai-itai-byo, or "ouch-ouch disease," in Japan was the historical event that for the first time drew the world's attention to the environmental hazards of Cd poisoning. In 1945, Japanese farmers living downstream from the Kamioka Zinc-Cadmium-Lead mine began to suffer from pains in the back and legs, with fractures, decalcification, and skeletal deformation in advanced cases.[26] The disease was correlated with the high Cd concentrations in the rice produced from rice paddies irrigated by contaminated stream water. The drinking water of the residents was also highly polluted.

The increased use of Cd and emissions from its production, as well as from Pb and steel production, burning of fossil fuels, use of phosphate fertilizers, and waste disposal in the past several decades, combined with Cd's long-term persistence in the environment, have reinforced the concerns first aroused by itai-itai-byo. Indeed, many researchers consider Cd to be one of the most toxic trace elements in the environment. Plants, animals, and humans are exposed to the toxicity of this metal, in different but similar ways. Like other heavy metals, Cd binds rapidly to extracellular and intracellular proteins, thus disrupting membrane and cell function.[27]

12.3.2 Characteristics and Use of Cadmium

Cadmium is a nonessential trace element and is present in air, water, and food. It is a silver-white metal with an atomic weight of 112.4, and a low melting point of 321°C. As a metal, Cd is rare and not found in a pure state in nature. It is a constituent of smithsonite ($ZnCO_3$) and is obtained as a byproduct from the smelting of Zn, Pb, and Cu ores.

A distinctive characteristic of Cd is that it is malleable and can be rolled into sheets. The metal combines with the majority of other heavy metals to form alloys. It is readily oxidized to the +2 oxidation state, resulting in the colorless Cd^{2+} ion. Cadmium has an electronic configuration similar to that of Zn, which is an essential mineral element for living organisms. However, Cd has a greater affinity for thiol ligands than does Zn. It binds to sulfur-containing ligands more tightly than the first-row transition metals (other than Cu), but Hg and Pb both form more stable sulfur complexes than does Cd. The Cd^{2+} ion is similar to the Ca^{2+} ion in size and charge density. About two thirds of all Cd produced is used in the plating of steel, Fe, Cu, brass, and other alloys, to protect them from corrosion. Other uses include solders and electrical parts, pigments, plastics, rubber, pesticides, and galvanized iron. Special uses of Cd include aircraft manufacture and semi-conductors. Because Cd strongly absorbs neutrons, it is also used in the control rods in nuclear reactors. Cadmium persists in the environment and has a biological half-life of 10 to 25 years.

12.3.3 Exposure to Cadmium

12.3.3.1 Airborne Cadmium

Human exposure to Cd occurs both in the occupational and general environment. Occupational exposure arises mainly from inhalation of contaminated air in some industrial workplaces. A variety of industrial activities can lead to Cd exposure. Some examples include mining and metallurgical processing, combustion of fossil fuels, textile printing, application of fertilizers and fungicides, recycling of ferrous scraps and motor oils, and disposal and incineration of Cd-containing products. Although aerial deposition is an important route of mobility for Cd, ambient air is not a significant source of Cd exposure for the majority of the U.S. population. In areas where there are no industrial facilities producing Cd pollution, airborne Cd levels are around 1 ng/m^3. This indicates that on average an adult may inhale approximately 20 to 50 ng of Cd daily.

Tobacco smoke is one of the largest single sources of Cd exposure in humans. Tobacco in all of its forms contains appreciable amounts of the metal. Because the absorption of Cd from the lungs is much greater than from the gastrointestinal tract, smoking contributes significantly to the total body burden. Each cigarette on average contains approximately 1.5 to 2.0 μg of Cd, 70% of which passes into the smoke.

12.3.3.2 Waterborne Cadmium

Cadmium occurs naturally in aquatic systems. Although it does not appear to be a potential hazard in open oceans, in freshwaters and estuaries accumulation of Cd at abnormally high concentrations can occur as a result of natural or anthropogenic sources. In natural freshwater, Cd usually occurs at very low concentrations (< 10 ng/l), however, the concentrations vary by area. Cd levels area also affected by environmental pollution; many Cd-containing wastes end up in lakes and marine water. Wastes from Pb mines, motor oils, rubber tires, and a variety of chemical industries are some examples.

The amount of Cd suspended in water is determined by several factors, including pH, Cd availability, carbonate alkalinity, and concentrations of Ca and Mg. Anions such as Cl^- and SO_4^{2-} may complex with Cd^{2+} ions, but this possibility is small in well-oxygenated freshwater. In waters low in organic carbon and other strong complexing agents, such as aminopolycarboxylic acids, free Cd^{2+} ions dominate the dissolved species.[28]

There is a distinct difference between the forms of Cd in marine waters and in freshwaters. In seawater, over 90% of the Cd is in the form of chloride salt ($CdCl_2$), while in river water Cd^{2+} is present mostly as $CdCO_3$.[29]

12.3.3.3 Cadmium Pollution of Soils

Cadmium pollution of soils can occur from several sources, including rainfall, dry precipitation, the deposition of municipal sewage sludge on agricultural soils, and the use of phosphate fertilizers. In acidic soils, Cd is more mobile and less likely to become strongly adsorbed to sediment particles of minerals, clays, and sand. Cd adsorption depends on the Cd concentration, pH, type of soil material, duration of contact, and the concentrations of complexing ligands.

12.3.3.4 Cadmium in Food

Cadmium exposure in the general environment comes mainly from food. Food consumption accounts for the largest source of Cd exposure by animals and humans, mainly because plants can bioaccumulate the metal (Table 12.1). Leafy vegetables, grains, and cereals often contain particularly high amounts of Cd (Table 12.2). Dietary intakes of Cd in uncontaminated areas of the world are in the range of 10 to 50 μg/day, whereas in contaminated areas the intakes may reach as high as 200 to 1000 μg/day.[30]

Aquatic organisms can potentially accumulate large amounts of Cd, therefore animals that feed on aquatic organisms may also be exposed to the metal. Birds may be exposed to high levels of Cd as they feed on grasses and earthworms in soils treated with municipal sludge.

12.3.4 Metabolism of Cadmium

Although dietary intake is the means by which humans are most highly to be exposed to Cd, inhalation of Cd is more dangerous than ingestion. This is because through inhalation, the organs of the body are directly and intimately exposed to the metal. Furthermore, 25 to 40% of inhaled Cd is retained, while only 5 to 10% of ingested Cd is absorbed (Figure 12.3). Following absorption, Cd appears in the blood plasma, bound with albumin.[31] The bound Cd is quickly taken up by tissues, preferentially by the liver. The Cd in the liver apparently cycles, bound with metallothionein (MT), through blood, kidney, and, to a small extent, bone and muscle tissue[29,31] In Japanese quail fed oat grain grown on soil treated with municipal sludge, bioaccumulation was highest in the kidney, followed by liver and eggs.[32]

Table 12.1 Accumulation of Several Metals in Plants

	Concentration (ppm, dry weight)		
Metal	**Soil**	**Plant**	**Plant:soil ratio**
Pb	10	4.5	0.45
Zn	50	32	0.6
Cd	0.06	0.64	10

Table 12.2 Cadmium Content of Selected Foods

Type of food	Cd content (μg/g wet weight)
Dairy products	0.01
Milk	0.0015–0.004
Wheat flour	0.07
Leafy vegetables	0.14
Potatoes	0.08
Garden fruits and other fruits	0.07
Sugar and adjuncts	0.04
Meat, fish, poultry	0.03
Tomatoes	0
Grain and cereal products	0.06

Excretion of Cd in mammals seems to be minimal under normal exposure. Miniscule amounts are excreted in the feces, and an immediate 10% excretion may occur in the urine. The half-life of Cd is about 7.4 to 18 years, and the long-term excretion rate is only 0.005% per day, beginning after about 50 years of age.[33]

12.3.5 Cadmium Toxicity

12.3.5.1 Toxic Effects on Plants

Plant exposure to Cd occurs through air, water, and soil pollution. Cadmium is highly toxic to plants; effects of toxicity include stunting, chlorosis, necrosis, wilting, and depressed photosynthesis. Because of leaf surface area, leafy plants may receive large amounts of Cd from the atmosphere. Plants are also greatly affected by high concentrations of Cd through waste streams from industrial facilities and from the use of sewage sludge as an agricultural fertilizer.

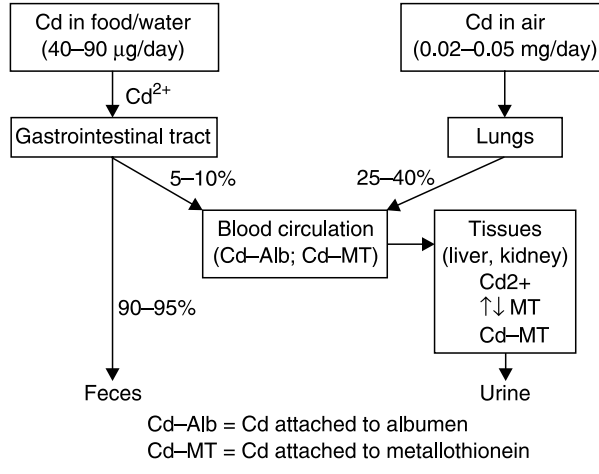

Figure 12.3 Cadmium metabolism in humans.

All plants can accumulate Cd, but the extent of accumulation varies with plant species and variety. Spinach, soybean, and curly cress, for instance, are sensitive to Cd, whereas cabbage and tomato are resistant. Tobacco plants have been shown to absorb high levels of Cd from the soil.[34] Several factors affect Cd uptake from soils, such as soil pH, organic matter, and cation exchange capacity. Of these factors, soil pH is the most important, with lower pH favoring uptake. Presence of soil organic matter and some minerals, such as chloride, also affect Cd uptake.

In higher plants, accumulation of heavy metals in the leaves is associated with a reduction in net photosynthesis. Cd primarily affects the photosynthetic pigments. Other studies also indicate inhibition by Cd of cellular functions in plants, such as photophosphorylation, ATP synthesis, mitochondrial NADH oxidation, and electron-transport system.

Cadmium inhibits seed germination under laboratory conditions.[1,12,13] Seedlings exposed to Cd solutions exhibit decreased root elongation and growth. The effect of Cd on seed germination, however, depends on several factors, including plant species. Cd was not found to be very toxic for germination and root growth of *Sinapis alba* seeds,[1] but the metal proves highly toxic to mung bean (*Vigna radiata*) seeds. For example, exposure of one-day-old seedlings to 10 and 50 μM $CdCl_2$ for 72 hours caused decreases in the fresh weight of radicles (hypocotyls and roots) by 7% and 13%, respectively. In addition, a general decrease in soluble sugar contents of the radicles occurred in the experimental seedlings. The activity of invertase, the enzyme responsible for the breakdown of sucrose to glucose and fructose in the rapidly growing roots, was decreased by 21% and 32% in seedlings exposed to 10 and 50 μM $CdCl_2$ for 72 hours, respectively.[35]

12.3.5.2 Effects of Cadmium on Animals

Cadmium toxicity in animals is mostly due to the ingestion of plant matter or to secondary poisoning from ingesting small prey exposed to high levels of the metal. Animals chronically exposed to Cd may exhibit emaciation, with a staggering gait, and rough hide-bound skin, stringy salivation, and lacrimation. Under microscopic observations, the trachea, rumen, and spleen may show abnormal cellular structure. The trachea may show complete sloughing of its epithelium, exposing underlying submucosa. In addition, stunted epithelial lining in the bronchi and bronchioles can occur. The renal glomeruli may be shrunken due to the capillaries. In some studies marked lymphocyte depletion in the spleen has been observed.

The toxicity of Cd to aquatic organisms is somewhat different. In seawater, various Cd binding ligands occur, and these appear to prevent Cd toxicity to any appreciable extent. The ligands may be derived from proteins, alginates, polyphosphates, and nucleotides resulting from tissue breakdown. In freshwaters, the liganding compounds may be provided by humic and fulvic acids from soil breakdown, citric acid, and synthetic chelating agents, often in

detergents from industrial sources. The ability of these ligands to bind Cd determines Cd toxicity in aquatic systems.

Other factors affecting Cd uptake into the tissues of aquatic organisms include salinity and temperature. A decrease in salinity causes an increase in the rate of Cd uptake. The apparent reason for this is that as salinity decreases, so does the Ca concentration of the water. Ca content of the water influences its osmolarity, which in turn affects Cd uptake. Temperature also affects Cd^{2+} absorption: when temperature increases, so does Cd^{2+} uptake.[29] The effects of salinity and temperature appear to be additive. The presence of some synthetic chelating agents affects the uptake of free Cd in aquatic organisms such as trout. The transfer of free Cd in chelate-free waters via fish gills is 1000 times greater than that complexed with EDTA.[36]

Because of their aquatic embryonic and larval development, and their sensitivity to a wide variety of toxicants, amphibians have often been used in studying environmental contamination.[37,38] In one study, the susceptibility of *Xenopus laevis* to Cd was examined during various developmental stages by exposing the embryos to varying levels of Cd^{2+}, ranging from 0.1 to 10 mg/l for 24, 48, and 72 hours. Results showed that malformations occurred at all developmental stages evaluated. The most commonly observed symptoms include reduction in size, incurvated axis, underdeveloped or abnormally developed fin, and abnormally small head and eyes.[38]

12.3.5.3 Effects of Cadmium on Humans

Human exposure to Cd occurs from airborne emissions, ingestion of contaminated foods, and through smoking. The adverse health effects caused by ingestion or inhalation of Cd include renal tubular dysfunction due to high urinary Cd excretion, high blood pressure, lung damage, and lung cancer. Cd and Cd compounds are "known to be human carcinogens," based on evidence of carcinogenicity in humans, including epidemiological and mechanistic information that indicate a causal relationship between exposure to Cd and Cd compounds and human cancer. In several cohort studies involving workers exposed to various Cd compounds, the risk for death from lung cancer is elevated.[20] A life-long inhalation of air containing 1 $\mu g/m^3$ is associated with lung cancer in about two subjects in 1000. Studies of long-term inhalation of $CdCl_2$ (12.5 to 50 $\mu g/m^3$) by rats showed a dose–dependent increase in the occurrence of lung cancer.

The gastrointestinal tract is the major route of Cd uptake in both humans and animals (Figure 12.3). The toxicity of the metal lies in that, after absorption, it accumulates in soft tissues, where it causes damage, as well as in the skeletal system. Furthermore, Cd accumulation in animals and humans occurs throughout their life spans. For example, in humans the Cd body burden at birth is only about 1 μg, at 6 years of age it is about 0.5 mg (500 μg), and at 64 years of age it is about 9.6 mg (Figure 12.4). Acute Cd inhalation (> 5 mg/m^3 in air), although rare, may lead to pneumonitis and pulmonary

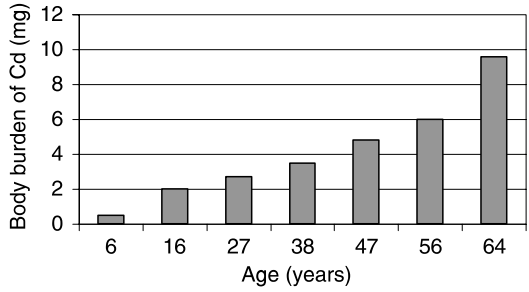

Figure 12.4 Cadmium accumulation with age in humans.

edema. Chronic exposure via inhalation may cause emphysema and chronic pulmonary effects.

The sites of greatest Cd accumulation are the liver and kidney. After inhalation or absorption from the gastrointestinal, Cd is concentrated in the kidney, where its half-life may exceed 10 to 20 years. One of the most widely known toxic effects manifested by Cd poisoning is nephrotoxicity. Although acute Cd exposure through ingestion of food contaminated with high levels of the metal can lead to proteinuria, this is rare. Adverse renal effects are more commonly seen with exposure to low levels of Cd. The effects are manifested by excretion of low-molecular-weight plasma proteins, such as β_2-microglobulin and retinol-binding protein (RBP).

The widely reported Cd poisoning itai-itai-byo episode occurred in Japan after World War II. The disease was caused mainly by ingestion of Cd-contaminated rice produced from rice paddies that had received irrigation water contaminated with high levels of the metal. Subsequent studies showed that persons with low intakes of Ca and vitamin D were at a particularly high risk.[39]

According to Nordberg,[31] several mechanisms may be involved in tubular Cd nephrotoxicity. It is assumed that the rate of influx of Cd-metallothionein (Cd-MT) into the renal tubular cell compartment on the one hand, and the rate of *de novo* synthesis of MT in this compartment on the other hand, regulate the pool of intracellular free Cd ions that can interact with cellular membrane targets in the tubules. When there is efficient MT synthesis but influx of Cd-MT into the lysosomes is limited, the free Cd pool is limited – no membrane damage occurs and Ca transport in the cell is normal. When Cd-MT influx into the lysosomal compartment is high and *de novo* synthesis of MT is deficient, the free Cd pool becomes sufficiently large to interact with membrane targets and block Ca transport routes. Under this condition, there is insufficient uptake and transport of Ca through the cell, leading to increased excretion of Ca and proteins in urine.

The excretion of Cd appears minimal under normal exposure. Loss in the urine is the major route of Cd excretion, while only minute amounts are excreted in the feces. As mentioned above, absorbed Cd persists in body

tissues. The long-term excretion rate of Cd is only 0.005% per day, beginning after about 50 years of age.[33]

A number of steps have been taken to protect humans from excessive Cd exposure. The EPA has established limits on the quantity of Cd that can be discharged into water or disposed of in solid wastes from factories that manufacture or use the metal. The EPA has established an interim maximum contaminant level for Cd in drinking water of 0.01 mg/l and has proposed a maximum contaminant level goal of 5.0 μg/l. The Occupational Safety and Health Administration has established average and maximum permissible exposure limits for Cd in workplace air at 200 $\mu g/m^3$ for dust and 100 $\mu g/m^3$ for fumes. These regulations will not only help to stop human exposure to Cd, but will also cut down on the exposure of plants and animals along the food chain.

12.3.6 Biological Effects of Cadmium

Cadmium has been shown to impair many plant cellular functions, such as ATP synthesis, succinate oxidation, photophosphorylation, mitochondrial NADH oxidation, and electron transport.[40] Cadmium is a potent enzyme inhibitor, affecting a variety of plant enzymes, such as PEP carboxylase, lipase, and invertase.

In humans and animals, Cd inhibits alkaline phosphatase and ATPases of myosin and pulmonary alveolar macrophage cells. Cd appears capable of inhibiting Phase I and Phase II xenobiotic biotransformation (Chapter 4) in the liver and kidney of rainbow trout. Hemoglobin concentrations in fish exposed to Cd are decreased, leading to anemia and liver damage. Inhibition of protein synthesis, enzyme activity, and competition with other metals are other deleterious effects of Cd on aquatic organisms.[29,33]

Two mechanisms appear to be involved in enzyme inhibition by Cd: one is through binding to –SH groups on the enzyme molecule, as is the case with Pb and Hg, the other is through competing with Zn and displacing it from metalloenzymes. Like other heavy metals of concern, Cd can also bind with SH-containing ligands in the membrane and other cell constituents, causing structural and functional disruptions. For instance, by inducing damage to mitochondria, Cd can uncouple oxidative phosphorylation and impair cellular energy metabolism. Induction of peroxidase activity by Cd in tissues of *Oryza sativa*, mentioned above, suggests the occurrence of Cd-dependent lipid peroxidation resulting in membrane damage. As discussed in Chapter 4, membrane damage due to lipid peroxidation is mediated by oxygen radicals and induction of peroxidase, superoxide dismutase (SOD), and catalase.

Interest in the defense response of organisms acutely exposed to Cd is growing. Plants, algae, and bacteria respond to heavy-metal toxicity by inducing different enzymes, creating ion influx or efflux to maintain ionic balance, and synthesizing small peptides. These peptides bind metal ions and reduce toxicity. Some plant species exposed to Cd and some other heavy metals produce a class of sulfur-rich polypeptides termed *phytochelatins* to complex,

and thus neutralize, the metals. According to Rauser,[41] phytochelatins act by directly binding to metal ions through chelation to form mercaptide complexes. Reddy and Prasad,[42] for instance, observed formation of a callus in plants exposed to Cd. The plants had higher protein content than the control plants. Over 200 plant species have been found capable of phytochelatin formation.

12.3.7 Cadmium and Nutrition

A close relationship exists between Cd toxicity and nutrition. For example, at moderate levels, Cd can antagonize several essential metals, such as Zn, Cu, selenium (Se), and Fe. The effect of Cd on mammals is thus influenced by the relative intakes of these and other metals.[43] Cadmium has been shown to decrease Zn content of serum and adversely affect serum insulin levels and glucose tolerance. This latter effect can be prevented in rats by increased Zn intake.[44]

Fe deficiency can influence Cd toxicity. Cd uptake by the body is increased during Fe deficiency or anemia. In mice, Cd has also been shown to compete with Fe in their transport systems. Studies on Fe absorption in mice receiving Cd in their drinking water showed that Fe absorption was significantly inhibited at a Cd dose of 1 mg/ml.[45]

The effect shown in laboratory mice has also been observed in humans. Mild anemia commonly occurs among industrial workers exposed to Cd dust fumes. Concern is also growing over the general population's exposure to Cd as levels in the environment, particularly in highly industrialized areas, have increased over the past several decades. As mentioned previously, Cd, once absorbed, is not readily excreted. With a long biological half-life in humans, concentrations of Cd may eventually become high enough to inhibit Fe absorption. This possibility is of particular concern because Fe deficiency is one of the most common nutritional problems in the world.

Newborn and young animals have the highest Cd absorption rate of all ages. The mechanism for this in mammals appears to be related to the absorption of Cd through milk. Because young animals need Ca for their growth and development, high amounts of calcium-binding protein (CaBP) are produced. It is thought that Cd utilizes the same transport system as Ca, or at least inhibits its functioning. The effect of Cd on the CNS is attributed to displacement of Ca from its action sites in the neuromuscular junction by Cd.[29]

Dietary protein also affects the toxicity of ingested Cd. A low-protein diet may lead to an increased absorption of Cd and thus increased toxicity. MT synthesis is decreased under low-protein conditions. A low-protein diet may lower MT availability for binding free Cd, resulting in increased Cd toxicity. Cadmium has also been shown to be related to lipid peroxidation and a decrease in phospholipid content in rat brains.[45] Such lesions may account, in part, for observed Cd-induced neurotoxicity.

Another nutrient with an important role in Cd toxicity is ascorbic acid (vitamin C). Vitamin C and Fe supplementation markedly reduced Cd accumulation in various soft tissues of rats, resulting in lowered toxicity.[46] It

is believed that vitamin C enhances Fe absorption through reduction of Fe^{3+} to Fe^{2+} as well as through chelation with Fe^{3+}.

12.4 MERCURY

12.4.1 Introduction

Mercury (Hg) is the only common metal that is liquid at room temperature. It has a high specific gravity (13.6 times that of water), a relatively low boiling point (357°C), and a long liquid range (396°C). Hg expands uniformly over its liquid range which, coupled with the fact that Hg does not wet glass, has made the metal ideal for use in thermometers. Hg has the highest volatility of any metal, and its good electrical conductivity makes it exceptionally useful in electrical switches and sealed relays. Many metals dissolve in Hg to form amalgams (alloys).

Mercury is rare in the earth's crust (0.1 to 1 ppm) and is not widely distributed, but it is ubiquitous, being measurable in trace amounts in most foods and water. Hg has no known biological role and, because of its diversity of usage, is an industrial health hazard. It is a bioaccumulative metal that is fat soluble, and has many damaging effects on living organisms.

12.4.2 Extraction and Uses of Mercury

Although several forms of ore occur, the principal one is *cinnabar*, the red sulfide, HgS. The extraction of Hg from the sulfide ore is accomplished by roasting the ore in air or with lime, as shown below:

$$HgS + O_2 \rightarrow Hg + SO_2 \tag{12.2}$$

$$4HgS + 4CaO \rightarrow 4Hg + 3CaS + CaSO_4 \tag{12.3}$$

The resultant metal is condensed from the furnace gases.

While Hg has a long history of use among pre-industrial humans, it is also used extensively by modern industry, such as in the manufacture of Hg batteries and other electrical apparatus and in laboratory equipment. Many Hg compounds, particularly acetate, oxide, chloride, sulfate, and phosphate, are used as catalysts in industrial chemistry. Hg compounds are added to paints as preservatives. In addition, Hg is used in jewelry making, some manufacturing processes, and in pesticides. The light emitted by electrical discharge through Hg vapor is rich in ultraviolet (UV) rays, and lamps of this kind, in fused quartz envelopes, are widely used as sources of UV light, such as in UV spectrophotometers. High-pressure Hg-vapor lamps are widely used for street and highway lighting.

In the U.S., the largest user of Hg is the chloralkali industry, in which chlorine and caustic soda are produced by electrolysis of salt (NaCl) solution.

One method of producing chlorine uses a flowing Hg cathode. The Na^+ ions discharge at the Hg surface, forming sodium amalgam Hg-Na. The resultant amalgam is continuously drained away and, as it is treated with water, NaOH solution and Hg are produced:

$$\text{Hg-Na} \xrightarrow{H_2O} \text{NaOH (solution)} + \text{Hg} \tag{12.4}$$

12.4.3 Sources of Mercury Pollution

Mercury is a naturally occurring metal dispersed throughout the ecosystem. Hg contamination of the environment is caused by both natural and anthropogenic sources. Natural sources include volcanic action, erosion of Hg-containing sediments, and gaseous emissions from the earth's crust. The majority of Hg comes from anthropogenic sources. Mining, combustion of fossil fuels (Hg content of coal is about 1 ppm), transporting Hg ores, processing pulp and paper, incineration, use of Hg compounds as seed dressings in agriculture, and emissions from smelters are some examples. In addition, Hg waste is produced as a by-product of chlorine manufacturing plants and gold recovery processes, and is found in used batteries and light bulbs.

Gold mining in the Amazon in recent years has led to Hg pollution. Hg enters the environment during each of the two steps involved in acquiring the gold. First, the sediments are taken from river bottoms and land mining sites and forced through sieves. The sieves are coated with mercury – the Hg bonds with the gold, separating it from the rest of the material. A large amount of Hg remains in the leftover soil and is a threat to the environment when this soil is discarded. Second, the gold–mercury amalgam is heated to purify the gold by vaporizing the Hg. When carried out in an unsealed container, Hg vapor will be emitted into the atmosphere. The Hg evaporated or burned in these operations can travel long distances, with subsequent precipitation by tropical rainstorms, leading to water pollution. As rainwater is rich in Hg^{2+}species formed by oxidation of Hg gas, contamination of fish can occur even in remote areas.

12.4.4 Biotransformation of Mercury

Various forms of Hg are present in the environment. Conversion of one form of Hg to another occurs in sediment, water, and air, and is catalyzed by various biological systems. For example, Hg that has been released to the atmosphere and washed back down to earth in rainwater often finds its way through river systems to be eventually deposited to lakes and seas. Microorganisms then convert the elemental Hg into methylmercury, CH_3Hg^+ (MeHg) through a process called methylation. The MeHg thus formed may then begin to move up the aquatic food chain. Alternatively, it may be split in a reaction mediated by bacteria, as shown in Figure 12.5.

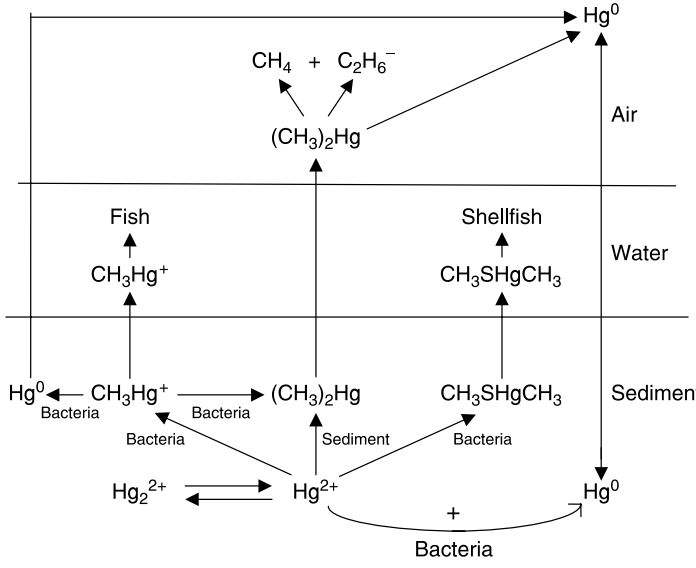

Figure 12.5 The mercury cycle, showing bioaccumulation of mercury in fish and shellfish. *Source*: NRC, *An Assessment of Mercury in the Environment*, 1978.

12.4.4.1 Biomethylation of Mercury

Soluble inorganic mercury salts can be converted to MeHg and dimethylmercury, $(CH_3)_2Hg$. This reaction can occur both aerobically and anaerobically. Alkyl cobalamines serve as alkylating agents, while methyl-B_{12} acts as a coenzyme in the reaction:

$$Hg^{2+} + 2RCH_3 \rightarrow (CH_3)_2Hg \rightarrow CH_3Hg^+ \qquad (12.5)$$

12.4.4.2 Demethylation of Methylmercury

The methyl group in $(CH_3)_2Hg$ may be split off to give rise to an Hg^{2+} ion and methane and ethane. The reaction is called *demethylation* and is catalyzed by two enzymes: a hydrolase and a reductase. The hydrolase hydrolyzes the mercury–carbon bond, yielding Hg^{2+} ions and methane. The reductase reduces the Hg^{2+} ion to metallic Hg. The Hg^{2+} ion may eventually be volatilized from the aqueous medium into the atmosphere. Demethylation appears to be much slower than methylation.

12.4.4.3 Methylmercury Biosynthesis and Diffusion into Cells

The rate of MeHg synthesis is determined by the microbial community and concentrations of soluble mercuric or mercurous species and methyl-B_{12} (which

acts as a coenzyme). The bioaccumulation of MeHg into the tissues of higher organisms, such as fish, appears to be controlled by diffusion. For example, MeHg-chloride diffuses through cell membranes into cells in 20×10^{-9} seconds. Once MeHg diffuses through the cell membrane, it is bound by –SH groups, thus maintaining the concentration gradient across the membrane, eventually leading to bioaccumulation. The mercury cycle demonstrating the bioaccumulation of Hg in fish and shellfish is depicted in Figure 12.5.

12.4.5 Toxicity of Mercury

12.4.5.1 Effects of Mercury on Algae

Very low concentrations of Hg can be lethal to some species of algae and impair the growth of others. Organomercurials have been shown to retard the growth and viability of several species of marine algae more effectively than inorganic Hg.[47] Concentrations of several alkylmercurial fungicides as low as 0.1 μg/l have been shown to decrease the growth and photosynthesis of some freshwater phytoplankton. The high sensitivity of phytoplankton to Hg compounds may be due to the high lipid content in the membranes or to the inhibition of lipid synthesis by the metal. Because phytoplankton is situated at the lowest trophic level in aquatic ecosystem, accumulation of Hg in phytoplankton can lead not only to disruption of the food chain, but also to bioaccumulation of the metal in organisms of higher trophic levels.

12.4.5.2 Effects of Mercury on Plants

All plants appear to concentrate traces of Hg. Total Hg levels in most common edible plants and foods derived from plants range from < 1.0 to 300 ng/g. The concentration of Hg in plants depends on Hg deposits in the soil, locality, plant species, the chemical form of the Hg, and soil aeration. Some plants have a barrier to the uptake and circulation of inorganic Hg salts and organically complexed mercurials adsorbed on clay, while others have no barrier against the uptake of gaseous Hg through the roots. In soils where decaying sulfides release gaseous elemental Hg, the vegetation may contain 0.2 to 10 μg/g (on a dry-weight basis).

Like Pb and Cd, Hg can cause deleterious effects on different species of plants. Hg is particularly toxic to barley plants, more so than Pb, Cr, Cd, Ni and Zn.[11] In rapidly dividing onion root cells, MeHg at $2.5 \times 10^{-7}\,M$ interferes with normal chromosome segregation by disrupting the mitotic spindle function.[48] Hg also impairs germination, as shown by depressed root elongation and shoot growth.

12.4.5.3 Effects of Mercury on Animals

Freshwater and marine organisms and their predators normally contain more Hg than do terrestrial animals, with the levels being highest in top predatory

fish. Fish may accumulate Hg in excess of the 0.5 mg/g FDA guideline. This accumulation is part of a dynamic process in which an organism strives to maintain equilibrium between intake and excretion. Numerous analyses have indicated that much of the tissue Hg in most fish is in the form of MeHg.[49] The Hg accumulated in fish comes primarily from absorption from the water across the gill or through the food chain, and some higher species may convert inorganic Hg into MeHg. Some Hg can also be taken up through the mucous layer and skin.

The metabolic rate of the fish and the Hg concentration in the aquatic ecosystem appear to be more important factors in bioaccumulation than age or exposure rate. Because increased temperature enhances metabolic rate, more Hg is concentrated in the summer than in the winter. The toxicity of Hg and other heavy metals to fish also increases with an increase in temperature. The 96-hour LC_{50} of Hg for freshwater crayfish (*Procambarus clarkii* [Girard]) was found to be 0.79 mg/l at 20°C, 0.35 mg/l at 24°C, and 0.14 mg/l at 28°C.[50]

Wild birds concentrate the highest levels of Hg in the kidney and liver, with less in the muscle tissues. Swedish ornithologists observed the first Hg-related ecological problems in the 1950s. Many species of birds declined, both in numbers and in breeding success, while Hg levels increased in the feathers of several species of seed-eating birds. In the U.S. and Canada, elevated levels of Hg were also found in seed-eating birds and their predators, presumably through eating Hg-treated seed. In 1970, both countries banned alkylmercurial seed dressings, and the Hg levels in game birds that do not feed on aquatic organisms decreased.

Age and diet markedly influence the rate of Hg absorption in animals. Suckling mammals have a high intestinal absorption rate due to their milk diet. Whole-body retention, high blood levels, and high accumulation in various organs, such as the brain, is seen in sucklings when compared with adult animals. For example, the absorption rate (as % of oral dose) of ^{203}Hg in one-week-old suckling rats was 38.2%, whereas in 18-week-olds on either a milk diet or a standard diet, the rate was 6.7% and 1%, respectively.[51]

The neurotoxicity of MeHg varies greatly between animal species. For example, nonhuman primates and cats metabolize MeHg similarly to humans, but rats and mice rapidly metabolize the compound to a less toxic inorganic form.[52]

12.4.5.4 Effects of Mercury on Human Health

Almost all the MeHg in the human diet appears to come from fish or other seafood, and possibly from red meat. Until recently, the Hg present in the atmosphere and in drinking water supplies was not considered to contribute significantly to the MeHg burden in human body. However, according to the EPA's risk assessment of human health, Hg is the toxin of greatest concern among 188 airborne toxins emitted from power plants. Coal-fired power plants are the largest source of anthropogenic Hg airborne emissions in the U.S. (40% of total emissions).[53] Some researchers consider there is a plausible link

between anthropogenic releases of Hg from industrial and combustion sources and MeHg in fish. In the U.S., 7.8% of women of childbearing age had blood levels of Hg exceeding the reference dose, the level at which most people could be exposed without risk.

The two major Japanese outbreaks of MeHg poisoning, in Minamata Bay and in Niigata, were caused by industrial discharge of MeHg and other Hg compounds into Minamata Bay and into the Agano River, resulting in accumulation of MeHg in fish and shellfish. The median total Hg level in fish caught in Minamata Bay at the time of the epidemic was estimated as 11 mg/g fresh weight. More than 700 cases of MeHg poisoning were identified in Minamata and more than 500 in Niigata.[54] (The Minamata Bay episode was caused by a chemical plant, called Chisso, which was manufacturing acetaldehyde using mercuric sulfate as a catalyst and discharging the waste, containing high levels of Hg, into the bay. Following the incident, the Chisso Corporation, then with 7000 employees, went bankrupt. The sediments contaminated with Hg were dredged, put into large steel drums, sealed, and buried at the bottom of the bay. Clean soils were then brought in to cover about 60% of the bay, converting it into a flat area of about 2×10^6 m^2. The cost for the project totaled about $300 million).

The critical organ concentration of MeHg may differ for different stages of the human life cycle. The developing fetal and newborn brain may be the most sensitive organ (i.e., critical organ) in terms of human MeHg toxicity. During the Japanese Minamata outbreak, 23 infants with severe psychomotor symptoms of brain damage were diagnosed. They were born to mothers who had consumed fish taken from the bay. The mothers were reported to have no symptoms or signs of MeHg poisoning other than mild paraesthesia (an abnormal sensation, as prickling, itching, etc.). It was concluded that MeHg had crossed the placenta and that the fetal brain was much more sensitive than the adult brain.

The largest outbreak of MeHg poisoning ever recorded occurred in Iraq during 1971 to 1972. The poisoning resulted from consumption of bread made from wheat that had been treated with a MeHg fungicide. It was reported that more than 6000 children and adults had been poisoned, with nearly 500 deaths. Symptoms observed among the victims include paraesthesis, ataxia, dysarthria, and deafness.[55] In this outbreak, an infant's blood Hg level was found to be higher than the mother's during the first few months of life, supporting the suggestion that the fetal brain is the critical organ in the exposed pregnant female.

The relative toxicity of various Hg compounds toward tissue depends on the relative ease of their formation of the Hg^{2+} ion. $HgCl_2$ is most toxic, while some nonionizable organic mercurials are relatively safe. Arylorganic Hg causes skin burns at high concentrations, while at low concentrations it may cause irritative dermatitis, but Alkylorganic Hg is most likely to accumulate in nervous tissue.

Inhalation of Hg vapor is perhaps the greatest source of danger in industrial and research laboratories. Hg vapor can diffuse through the alveolar

membrane and reach the brain, where it may interfere with coordination. Pronounced brain damage occurs in victims of Hg poisoning.

The biological half-life of Hg is estimated to be 70 days. The critical daily intake has been estimated to be 300 mg Hg as MeHg for an average 70-kg man.

Chronic Hg poisoning may result from exposure to small amounts of Hg over long periods, such as may occur in industries that use Hg or its salts. The symptoms include salivation, loss of appetite, anemia, gingivitis, excessive irritation of tissues, nutritional disturbances, and renal damage accompanied by proteinuria. Acute Hg poisoning results from ingestion of soluble Hg salts. Mercuric chloride precipitates all proteins with which it comes into contact. Vomiting usually occurs a few minutes after ingestion. The victim experiences extreme salivation and thirst, nausea, severe gastrointestinal irritation, and abdominal pain. Loss of fluids and electrolytes occurs.

Chemists and biologists across the U.S. were shocked in the summer of 1997 by the death of Dartmouth College chemistry professor Karen E. Wetterhahn as a result of acute exposure to dimethylmercury.[56] It was reported that she was apparently transferring dimethylmercury in a fume hood when 0.1 to 0.5 ml of the compound spilled on the disposable latex gloves she was wearing and permeated them, quickly seeping into her skin. She became ill a few months later and died of Hg poisoning less than a year after the exposure.

12.4.6 Biological Effects of Mercury

Mercury, like many other heavy metals, is extremely toxic because as an ion or in certain compounds it is soluble in water. For this reason it is readily absorbed into the body, where it tends to combine with and inhibit the functioning of various enzymes. The ultimate effects of Hg in the body are similar to those of Pb and Cd: inhibition of enzyme activity and cell damage. Hg has been reported to inhibit a large number of enzyme systems.[57] The particular reactivity of Hg with thiol ligands has further confirmed the selective affinity of this metal to react with –SH group, as shown in the following example with MeHg:

$$RSH + CH_3Hg^+ \rightarrow R\text{-}S\text{-}Hg\text{-}CH_3 + H^+ \qquad (12.6)$$

Mercury is known to affect the metabolism of mineral elements, such as sodium (Na) and potassium (K), by increasing their permeability. Hg also:

- inhibits the active transport mechanism through dissipation of the normal cation gradient
- destroys mitochondrial apparatus
- causes swelling of cells, leading to lysis
- decreases α- and γ-globulins while increasing β-globulin, suggesting liver dysfunction
- decreases DNA content in cells and adversely affects chromosomes and mitosis, leading to mutagenesis

Exposure of rat lung cultures to low concentrations of Hg^{2+} ions (added as $HgCl_2$) appears to be cytotoxic as it alters the rates of DNA, RNA, and collagen synthesis. For example, exposure to 0.1 to 10.0 μM Hg^{2+} ions increased DNA synthesis by 2.5 to 3.5 times after 24 hours, but the rate decreased over the 5-day culture period.[58]

The MT protein receptor present in kidney tissue tends to bind actively with Hg and may thus exercise a protective effect. When the MT receptors are saturated with Hg, morphologic damage becomes manifest. An adaptive mechanism may also exit; MT content in the kidneys increases with repeated Hg exposure.

12.4.7 Mercury and Nutrition

Dietary Se has been shown to exhibit a protective effect against Hg toxicity.[59] Treatment with Se reduced the lethal and neurotoxic effects of MeHg and other Hg compounds. The reason for this protective action is not clear. The interaction of MeHg with –SH groups is considered to be the natural biological sink for the Hg compound. Approximately 95% of the Hg bound to fish protein has been shown to be part of MeHg–cysteinyl coordination complex. The selenohydryl group has been shown to bind MeHg 100 times more tightly than the –SH group.[60] In addition to Se, vitamin E is also known to protect against the toxic effect of MeHg. However, a much higher concentration of this vitamin is required to provide the same level of protection.

Ascorbic acid added to the diet of chicks was shown to overcome growth depression caused by 500 ppm Hg. It was shown that ascorbic acid had an effect on Hg metabolism that was not mediated through Fe metabolism – adding 1000 ppm Fe in the diet did not overcome the growth depression caused by Hg. High dietary levels of ascorbic acid might result in increased urinary excretion of cations to balance the excretion of the ascorbate anion and thus increase the rate of excretion of toxic elements.

In the U.S., fish consumption has increased considerably (ca. 25%) over the past decade. This increase is mainly attributed to general knowledge about the nutritional value of fish, including, for example, its high protein content, relatively low levels of calories, cholesterol, and fats, particularly saturated fats, and its high levels of ω-3 fatty acids. Some researchers, however, are concerned about the trend of increased fish consumption because with increased seafood consumption comes increased risk of exposure to highly toxic MeHg.

12.5 NICKEL

12.5.1 Introduction

Nickel (Ni) is a white metal, with a faint tinge of yellow. Although it is the fifth most abundant element in the biosphere, Ni was only discovered through the

mining of other metals. Its principal ores are nickelite (NiAs), millerite (NiS), and pentlandite ([Ni,Fe]S). Ni is quite mobile through the air, water, and soil. Historically, the focus of concern about this metal was how to increase occupational safety, but many researchers are now paying more attention to examining nickel's role in the health of ecosystems.

Nickel was largely ignored for industrial use until just before 1900, when the Mond carbonyl process was discovered as a way to remove the metal in a pure form from the mined ores. This process was the key to triggering concern for worker safety because part of it involved nickel carbonyl gas, $Ni(CO)_4$, the most toxic form of the metal. Other forms of Ni, however, play an uncertain role in the safety of workers and the public. Overall demand for Ni has been increasing over time, mostly due to increasing stainless-steel production. Ni is used in approximately 250,000 industrial applications, and is used in the forms of nickel carbonate, nickel carbonyl, nickel chloride, nickel nitrate, nickel oxide, nickel sulfate, and nickel sulfide.[61] Some applications include use in iron processing, nickel plating, and nickel–cadmium batteries. Nickel iron is used for electrical equipment, copper nickel is used as an anticorrosive for marine vessels and equipment, and nickel titanate is used as a pigment in paints. As Ni refineries increase production, the concern for this heavy, mobile metal and its effects on the environment also increases.

12.5.2 Sources of Environmental Nickel Pollution

Environmental contamination by Ni occurs naturally and anthropogenically. The natural sources include volcanoes, ocean spray, soil dust, and forest fires, with a particulate size ranging from 2 to 10 μm. Examples of anthropogenic sources include the mining, smelting, and refining of Ni, with release of much smaller size of particulate matter (0.1 to 2.0 μm). Ni is released from the melting of stainless steel during scrap recycling. Other forms of mining, including gold mining, may release Ni into the surrounding environment as a by-product, usually with other leachates. Nickel sulfate is released in the burning of fossil fuels and sewage incineration. Sources of Ni air pollution include, in addition to those mentioned earlier, processing of Ni, burning of petroleum products, and plastic production. The concentrations of Ni in the air are increased over industrialized areas. For example, the highest concentration of the metal in the U.S. is found in South Carolina, with 116 ng/m^3 of air.[40]

An elevated Ni concentration is often found in the water of lakes near industrial areas. Nickel–cadmium batteries are also a potential source of Ni water pollution, which may result from leaching from waste sites.

12.5.3 Health Effect of Nickel

The most common type of Ni exposure for the public is through direct skin contact with Ni plating. $Ni(CO)_4$ gas, the most toxic of the Ni compounds, was the first to cause deaths in refineries. In April 1953, the Gulf Oil Company in

Port Arthur, Texas, exposed more than 100 workers to $Ni(CO)_4$ gas during repair work. Two workers died at the scene and 31 were hospitalized. The immediate symptoms included headaches, nausea, weakness, dizziness, vomiting, and epigastric pain. There was a latency period of 1 to 5 days, followed by secondary symptoms, which included chest constriction, chills and sweating, shortness of breath, coughing, muscle pains, fatigue, gastrointestinal discomfort, and, in severe cases, convulsions and delirium.

Nickel carbonyl is a volatile liquid with extraordinary toxicity, particularly to the lungs. It is an intermediate produced in refining nickel ore. The mechanism of this toxicity is not known, but the lungs play a major role in both absorption of $Ni(CO)_4$ vapors and excretion of parenterally administered $Ni(CO)_4$. Sixty minutes after intravenous administration of ^{14}C- or ^{63}Ni-labeled $Ni(CO)_4$, 25% was exhaled unchanged, 11% was exhaled as ^{14}CO (with only traces of $^{14}CO_2$ detected), 10% was present as ^{14}C-carbon-monooxyhemoglobin, and 6.5% was present as unchanged $Ni(CO)_4$ in whole blood. Translocation of ^{63}Ni from erythrocytes to plasma correlated with the disappearance of $Ni(CO)_4$ from whole blood.

Since metal–CO bonds are greatly weakened by oxidation of the central metal, it is conceivable that $Ni(CO)_4$, like Hg^o, might be biotransformed *in vivo* by the catalase–H_2O_2 system, as outlined in Reaction 12.7:[62]

$$Ni(CO)_4 \xrightarrow[(enzyme)]{-2e} [Ni(CO)_4^{2+}] \xrightarrow[\text{fast}]{} 4CO + Ni^{2+} \qquad (12.7)$$

Worker exposure also occurs through inhalation of Ni dust formed in the refining process, through grinding, calcination, and leaching of the metal ore. Ni, especially insoluble forms such as nickel oxides, nickel subsulfide, and metallic nickel, has been hypothesized as a possible carcinogen, which prompts carcinomas 20 to 35 years after original and consistent exposure. Symptoms may include nasal boils, cysts, perforation of the nose, pharyngitis, sinusitis, and nasal polyps. Since humans excrete the majority of the Ni they are exposed to, the concern for the public is not substantial.

The safety level set by the Occupational Safety and Health Administration for $Ni(CO)_4$ gas is 7 $\mu g/m^3$. No safety level has been set for the concentration of Ni in drinking water. Exposure to nickel sulfate and nickel chloride in drinking water can cause vomiting and headaches for up to 5 days,[63] and the metal can cross human placental barrier, affecting the fetus.

The ionic form of Ni can compete with Ca, cobalt (Co), Cu, Fe, and Zn in compounds, so there is a possibility of interference with processes such as Fe absorption. Ni is inhibitory to a number of enzymes in the human body, including urease and carbon monoxide dehydrogenase. Ni-sensitive individuals often develop contact dermatitis as a result of exposure to jewelry and metal snaps containing Ni. Workers who have increased exposure to Ni include cashiers, ceramic workers, electricians, electroplaters, hairdressers, jewelers,

mechanics, and metal workers or welders. Interestingly, women are reported to be four times more sensitive to Ni dermatitis than men.[64]

Inhalation of Ni compounds has been considered responsible for lung, sinonasal and laryngeal carcinomas in Ni refinery workers. In the 1930s, researchers found that these workers experienced 16 times the rate of lung cancer, and 11 times the rate of nasal sinus cancer compared with the surrounding population.[64] Other illnesses observed included pneumonoconiosis and emphysema. The tracking of the health of refinery workers demonstrated two different forms of Ni to which workers were exposed: $Ni(CO)_4$ gas and Ni processing dust. Ni may be reabsorbed in the kidney, possibly linking it to some forms of kidney cancer. Interestingly, among the different types of Ni compounds, particles (< 5 μm) of crystalline nickel subsulfide (Ni_3S_2) were carcinogenic, whereas those of the amorphous NiS were not. In a study using cell cultures, particles of the carcinogenic crystalline Ni_3S_2 were shown to be actively phagocytized by Syrian hamster embryo cells and Chinese hamster ovary cells, but the cells did take up significant quantities of similar-sized particles of the noncarcinogenic amorphous NiS.[65]

Magnesium has been shown as an effective protector against Ni-induced carcinogenesis *in vivo*. The mechanisms involved in the protective effect of Mg are unclear. Recent studies showed that DNA-protein crosslinks and chromosomal aberrations in mammalian cells were increased in cultures treated with Ni compounds.[66] In addition, Ni(II) caused oxidative damage to isolated DNA and chromatin in the presence of H_2O_2, suggesting the formation of reactive oxygen species. The genotoxic effects of Ni were lessened by added $MgCO_3$. Furthermore, the cells of fibroblasts used in the study showed an 80-fold increase in Ni levels following treatment with nickel subsulfide, but a decrease in the presence of $MgCO_3$. These results suggest that the protective role of Mg in Ni-induced cytotoxicity and genotoxicity can be attributed to its ability to reduce either the intercellular Ni concentration or reactive oxygen formation.[66]

12.6 ARSENIC

12.6.1 Occurrence and Properties of Arsenic

Arsenic (As) is a ubiquitous element present in various compounds throughout the earth's crust. It is a member of group VA of the periodic table and has the common oxidation states of -3, $+3$, and $+5$. The redox states of As are arsenate (H_3AsO_4), and arsenite (H_3AsO_3), which are present in soil solutions. Trivalent As (As^{3+}) is more soluble and mobile than the pentavalent form (As^{5+}).[67] Some microorganisms can oxidize or reduce As; these include strains of *Bacillus* and *Pseudomonas*.[67] *Penicillin brevicaule*, called the arsenic fungi, can produce toxic and highly volatile arsenes. Fungi, yeasts, and bacteria can methylate As into monomethylarsonate, dimethylarsinate, and gaseous derivatives of arsine, which are widely distributed in soils.[68] Methylation of

As to organoarsenicals also occurs in invertebrates and vertebrates, including humans.

Estimated levels of As in different sources are:

- seawater: 2 to 5 ppb
- public water supplies: ~5 ppb (recommended limit is 10 ppb)
- uncontaminated soil: ~5 ppm
- human food: plant sources: < 0.5 ppm
- fish/seafood: much higher

An estimated average dietary intake in the U.S. is about 0.9 mg/day, and the total body burden of As in adult is about 15 to 20 mg.

12.6.2 Uses of Arsenic

The use of arsenical compounds increased greatly during the 18th and 19th centuries. Arsenical compounds were preferred for the control of agricultural pests, before the widespread use of organochlorines and organophosphates.[69] For example, Paris Green ($[CH_3COO]_2Cu \cdot 3Cu[AsO_2]_2$) and lead arsenate ($PbHAsO_4$) were used as insecticides, white arsenic (As_2O_3) was used as rodenticide, sodium arsenite (a solution of NaOH and As_2O_3) as an insecticide and herbicide, and methylarsenic sulfide (CH_3AsS) as a fungicide. As compounds have also been used in various pharmaceutical substances. The use of As in veterinary medicine as a nutritional supplement and in the treatment of disease dates back to the 15th century.[70] For the past century, the chronic feeding of small doses of various As preparations has been reported to increase appetite, improve the level of activity, correct anemia, and improve the coats of animals. Arsenic was used as a feed additive to aid in the prevention and control of certain enteric diseases of swine and poultry and to improve weight and feed efficiency of livestock in general.[70] Other miscellaneous uses of arsenic include pigments and dyes, preservatives of animal hides, glass manufacture, and wood preservatives. Currently, veterinarians employ an organic arsenical, sodium capasolate, for the treatment of heartworms in dogs.[71]

12.6.3 Sources of Exposure to Arsenic

Arsenic can be emitted into the environment from several natural sources, including volcanic eruptions. Weathering and the processes of sedimentation lead to a wide, natural distribution.[72] Methylation by microorganisms mobilizes organoarsenic compounds in groundwater. As^{5+} is slightly more mobile in soil at pH 5.8, but as pH rises As^{3+} becomes more mobile than As^{5+} (although both increase in mobility).[70] Thought to be pollution-free and environmentally friendly, geothermal wells, used as a source of energy, are also a source of As for surface waters. Forest fire can disperse arsenicals to the wind.[72]

Combustion of fossil fuels and smelting of non-ferrous metals are unintentional pathways for release of arsenic, generally as arsenic trioxide, into the environment. As occurs in most coals, in association with sulfur. When burned, arsenic, along with other trace elements, accumulate on fly-ash particles. Although found only as trace amounts in coal, the amount present on fly ash is significant.[72]

Sulfur compound are often present in mine tailings, which can oxidize, leading to the formation of an acidic solution. This solution can dissolve many elements, including As, which may then leach into ground and surface waters.[72]

12.6.4 Human Exposure to Arsenic

Arsenic is present in urban air at levels of about 0.02 μg/m^3, and in soil at levels ranging from 0.2 to 40 μg/g. The standard for As in the U.S. water supplies, set by the EPA, is 50 μg/l. Although most drinking water supplies in the U.S. contain levels lower than 5 μg/l, about 350,000 people might consume water containing a higher level of As than the EPA standard.[73]

For the general population, the main exposure to inorganic As is through ingestion. Both organic and inorganic arsenics are present in varying amounts in food. Fish, for example, contains relatively high concentrations of organic arsenic. However, inorganic forms of arsenic can exist as either arsenate or arsenite. Although arsenate is less toxic, it can be converted to arsenite in humans through metabolism.

Arsine (AsH_3) is a colorless, non-irritating gas that is liberated from any arsenic-containing ore that is treated with an acid. Workers in smelters, ore refineries, and areas that are involved with galvanizing, soldering, etching, and Pb plating are at greater risk.[74]

12.6.5 Animal Exposure to Arsenic

Arsenic enters into animals through three routes: the respiratory tract, the gastrointestinal tract, and the skin. In ambient air, As exists mainly as an inorganic form but is also present at low levels in methylated forms.[75] The depth of penetration into the lungs depends on the size and chemical form of the As particles. Fly-ash particles are small enough to be deposited in the pulmonary region of the respiratory tract. Water-soluble forms of As, such as sodium arsenic and dimethylarsenic acid, are readily absorbed. Large As-laden airborne particles may be removed by the mucocilliary apparatus, only to be transported to and absorbed in the gastrointestinal tract.[75]

The solubility of As compounds determines the degree of absorption in the gastrointestinal tract. In general, greater than 90% of $As^{3+}$and As^{5+} given as a water solution is absorbed.[76,77] In experiments using cats and rats, As absorption is highest in the small intestine, and oral and gastric absorption is low. Absorption through skin is poorly documented. There are incidents

reported that indicate arsenic trichloride and arsenic acid are absorbed by the skin. Acute dermal injury by arsenic acid enhances absorption.[75]

12.6.6 Distribution of Arsenic in the Body

After absorption, inorganic arsenic is transported by blood to other organs. A single oral dose of inorganic arsenic results in elevated As concentrations in liver, kidneys, lungs, and intestinal mucosa.[77] In humans who died of acute arsenic poisoning, the highest As levels were found in the liver, kidney, intestinal mucosa, and spleen.[75]

The relatively even distribution of As, experimentally administered in different valence forms, is probably due to methylation in the liver and distribution as dimethylarsinic acid. If given at high doses, metabolism is saturated and inorganic forms are then distributed. Arsenic concentrations are high in skin, hair, and nails. Samples taken from these organs have often been used for exposure analysis. Administration of organic arsenicals to experimental animals results in high levels of arsenic in liver, kidney, and spleen. However, levels are lower in erythrocytes when compared with animals dosed with inorganic forms.[75]

Some As, predominantly in the pentavalent form, is excreted directly in feces. Once absorbed, As^{5+} is excreted by the kidneys. Trivalent arsenic is readily excreted in bile to the intestines, where it is available for reabsorption or fecal elimination.

12.6.7 Toxicity of Arsenic

12.6.7.1 Toxicity to Plants

Although research on the toxicity of As to plants is limited, available information indicates that As is potentially toxic to some plant species. For instance, Fargasova[1] studied the acute toxicity of five metals, including Cr^{6+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, and As^{5+}, on the germination of mustard seeds (*Sinapis alba*) and found that after 72 hours the most toxic metal for seed germination was As^{5+}. Similar results were obtained from laboratory experiments in which mung bean (*Vigna radiata*) seedlings were exposed to As^{3+}, As^{5+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, and Zn^{2+} for 72 hours. Arsenic proved to be far more detrimental to mung bean germination than other metals tested.[78]

12.6.7.2 Toxicity of Arsenic to Animals and Humans

The toxicity of As to mammals is related to its absorption and retention in the body, and varies with chemical form. The toxicity of arsenicals in decreasing order is: inorganic arsenites > organic trivalent compounds (arsenoxides) > inorganic arsenates > arsonium compounds > elemental arsenic.[79] Toxicity appears to be related to the solubility of the arsenical in water. The low toxicity of elemental arsenic is attributed to its near insolubility in water and body

fluids. Trivalent arsenic is much more toxic than pentavalent arsenic.[67] Arsenate is well absorbed and rapidly eliminated, mainly in urine. Arsenite is also well absorbed, but is retained in greater quantities and for longer periods in tissues.

Subsequent to absorption, methylation as well as interconversion between arsenate and arsenite occurs in the body. The interconvertion occurs via cytochrome c and cytochrome oxidase.[75,79] Specifically, arsenate (As^{+5}) is reduced to As^{+3}, which is then methylated to monomethylarseneic acid and then to dimethylarseneic acid. Methylation occurs mostly in the liver and is facilitated by the presence of S-adenosyl-methionine (SAM). The methylated metabolites are less toxic, less cytotoxic, and are more readily excreted in urine.

Arsenite orally administered to mice was found to cross the blood–brain barrier, leading to modified metabolism and function of the CNS, as evidenced by increase in the arsenic trioxide content in discrete brain areas, and increased metabolites of norepinephrine and dopamine in the cerebral cortex (but decreased in the corpus striatum). Metabolites of 5-hydroxytryptamine increased in all the discrete brain areas. The vertical and horizontal motor activity was increased.[80]

Capillary injury and dilatation also occur, resulting in transudation of fluid, which in turn decreases blood volume and causes circulatory collapse. Blackfoot, a disease endemic in Taiwan, is caused by As and characterized by the loss of circulation to the extremities, resulting in gangrene.[79] Arsenic induced capillary changes within the kidneys cause tubular degeneration.

Inorganic As compounds are ''known to be human carcinogens,'' based on sufficient evidence of carcinogenicity in humans.[20] Many cases of skin cancer have been reported among people exposed to As through medical treatment with inorganic As^{3+} compounds. An association between environmental exposure to As through drinking water and skin cancer has been observed and confirmed. Epidemiological studies in areas where drinking water contained As at levels of 0.35 to 1.14 mg/l showed increased risks of cancers of the bladder, kidney, skin, liver, lung, and colon, for both men and women. Occupational exposure to inorganic As, especially in mining and copper smelting, has consistently been associated with an increased risk of cancer. An almost ten-fold increase in the incidence of lung cancer was observed in workers most heavily exposed to As. Humans exposed to inorganic arsenicals may also have increased risk of cancers in lymph and hematopoietic tissues.[79]

Several populations are at risk of high incidence of skin cancer due to water contamination, particularly Chileans and some Taiwanese. Cancer is highest among elderly persons who show symptoms of chronic As poisoning. In general, the incidence of As-induced cancer is dramatically lower in animals.[79] However, As is known to be a teratogen in several species of animals, as well as in humans.

Several fungi and bacteria have been shown to methylate inorganic As. If the As is in the pentavalent form, it is reduced to arsenite and methyl groups are added. In animals, As^{5+} appears to be reduced to As^{3+} in the kidney. Methylation then occurs in the liver. Methylation is considered the major

detoxification mechanism for inorganic pentavalent arsenates and trivalent arsenites in mammals. Methylated arsenicals rapidly clear from all tissues, except the thyroid.[79]

Workers at the greatest risk include those in smelters and those associated with pesticides. Chromated copper arsenate (CCA) is the most common form of As used as wood preservative. There are concerns that As, as a known carcinogen, may leach from wood and expose children to unsafe levels. The EPA reached a voluntary agreement with industry to phase out the use of CCA to treat wood for residential structures by the end of 2003.

12.6.8 Biological Effects of Arsenic

Cells accumulate As by using an active transport system normally used in phosphate transport.[79] Once absorbed, As toxicity is generally attributed to the trivalent form. Toxic effects are exerted by As reacting with sulfhydryl enzyme systems.[70,79] The tissues rich in oxidative metabolism, such as the alimentary tract, liver, kidney, lung, and epidermis, are therefore most affected.

Like Pb, Cd, and Hg, discussed above, As is toxic to living organisms primarily because of its ability to react with and inhibit SH enzyme systems. Figure 12.6 shows the reaction between arsenite (AsO_3^{3-}) and two glutathione (GSH) molecules. The resultant strong covalent bond (–S–As–S–) effectively eliminates the GSH molecules from further reactions.

In animals, As^{5+} appears to be reduced to As^{3+} in the kidney. This is followed by methylation in the liver. Methylation is considered the major detoxification mechanism for inorganic arsenates and arsenites in mammals. Arsenite inactivates enzymes such as dihydrolipoyl dehydrogenase and thiolase, resulting in inhibition of pyruvate oxidation and β-oxidation of fatty acids.[79,81]

Arsenic, as As^{5+}, acts as an uncoupler of oxidative phosphorylation and substrate level phosphorylation associated with glycolysis. For example, a key step in glycolysis is the conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. An inorganic phosphate (P_i) participates in this reaction. Arsenate (AsO_4^{3-}), which closely resembles P_i in structure and reactivity, can replace phosphate in attacking the energy-rich thioester intermediate, as shown below:

$$\text{Glyceraldehyde 3-phosphate} + HAsO_4^{2-} \rightarrow \underset{\text{arsenophosphoglycerate}}{AsO_4\text{–GAc–P}} \qquad (12.8)$$

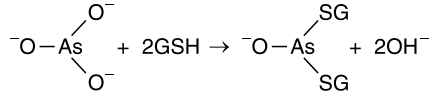

Figure 12.6 Interaction of arsenite with two molecules of glutathione (GSH).

The product of this Reaction 12.8, 1-arseno-3-phosphoglycerate, is unstable and rapidly hydrolyzed. Hence, in the presence of arsenate, the net reaction is:

$$\text{Glyceraldehyde 3-phosphate} + NAD^+ + H_2O \rightarrow \text{3-phosphoglycerate} + NADH + 2H^+ \quad (12.9)$$

An arsenic-containing poison gas called Lewisite was reported to have been used in World War I. Attempts by British scientists to develop a compound to counteract Lewisite led to an understanding of how As acts as a poison and subsequently to the development of an antidote. After recognizing that Lewisite poisoned people because of the reaction between As and the protein –SH group, the scientists set out to find a suitable compound that contained a highly reactive –SH group that could compete with the protein –SH groups for As, thus rendering the poison ineffective. The research culminated in the discovery of a compound known as British Anti-Lewisite (BAL). It is now known that BAL is a chelating agent and, as such, can react with some metal ions and As (Figure 12.7).

There has been a growing interest among biologists and biochemists in the As-induced oxidative stresses as a possible mechanism to explain some of the adverse effects observed with As toxicity. However, the mechanisms involved remain unclear. Zaman and Pardini[82] reported that both As^{3+} and As^{5+} alter the activities of SOD, catalase, glutathione S-transferase, and glutathione peroxidase. They subsequently have used the activities of these enzymes as markers for As toxicity tests for invertebrates.

Studies by Pi et al.[83] demonstrated that in humans, chronic exposure to high levels of As from drinking water elevated serum lipid peroxide (LPO) levels, and lowered non-protein sulfhydryl (NPSH) levels, suggesting oxidative stress. This observation was derived from epidemiological and biochemical studies on the residents from two villages in Inner Mongolia, China. Residents from the first group had been chronically exposed to 0.42 ppm inorganic As (iAs) in their water supplies (high-As-exposed group), whereas the second group had been consuming 0.02 ppm iAs in their water supplies (low-As-exposed group). The outcome of the study was that residents of the high-As-exposed group showed significantly increased LPO concentrations, but decreased NPSH levels, compared with those of the low-As-exposed group.

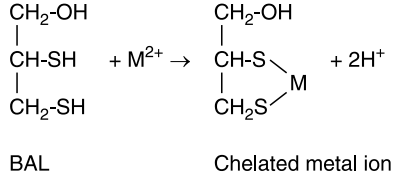

Figure 12.7 British Anti-Lewisite (BAL) chelation of a heavy metal ion.

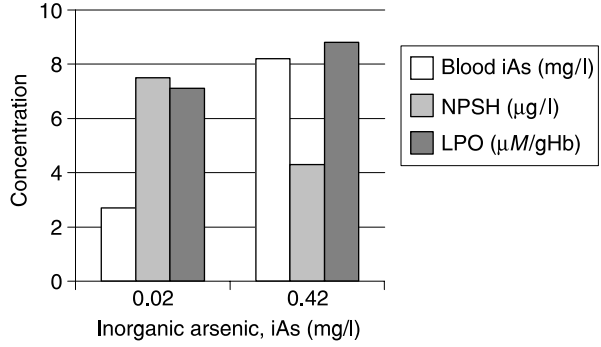

Figure 12.8. Effect of arsenic in drinking water in oxidative stress.
Source: Pi, J. et al., *Environ. Health Persp.*, 110, 331, 2002.

As expected, the serum iAs levels of the high-As-exposed group were much higher than those of the low-As-exposed group (Figure 12.8).

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12.8 REVIEW QUESTIONS

1. Which metals are generally considered the most toxic?
2. Which metal is designated by the EPA as one of the six "Criteria Air Pollutants?"
3. Why is lead shot a health risk for birds?
4. What is meant by a "systemic poison?"
5. Why are children more vulnerable to Pb exposure than adults?
6. What is the main reason that Pb is inhibitory to many enzymes?
7. Name the two enzymes involved in heme synthesis that are inhibited by Pb.
8. What is "ouch-ouch disease?" Briefly explain how it occurred.
9. What are the main adverse health effects that Cd has on humans?
10. What is the basis of Cd toxicity in humans and animals?
11. What is metallothionein? How is it related to Cd exposure in humans?
12. Describe the two mechanisms involved in enzyme inhibition by Cd.
13. What is phytochelatin? How does it act in plants?
14. Explain how dietary protein may be related to the toxicity of ingested Cd in humans.
15. Explain how biomethylation of Hg occurs.
16. Explain the toxicity associated with inhaled Hg vapor.
17. What is the most toxic form of Ni?
18. What types of cancer are considered to be related to Ni inhalation?

19. Explain the relationship between Mg and Ni.
20 Which is more toxic, arsenite or arsenate? Can arsenate be converted to arsenite in the body?
21. Which one of the following is most toxic to the germination of mustard seeds: Cd, Cr, As, Hg, or Pb?
22. How do As^{3+} and As^{5+} affect the antioxidant enzymes?
23. What is the importance of methylation of arsenites and arsenates in mammals?
24. How does As affect the phosphorylation process associated with glycolysis?
25. How is As toxicity related to phosphorus in the tissue?
26. What are Lewisite and anti-Lewisite?
27. How is As related to oxidative stress?

Chapter 13

Pesticides and Related Materials

13.1 INTRODUCTION

A pest, broadly defined, is any organism – plant, animal, or microorganism – that is destructive or troublesome, or living where it is unwanted. Pesticides refer to any chemicals intended to prevent, deter, destroy, or otherwise impair the ability of pests to compete with desired organisms, such as crops, animals, or humans. Pesticides can be classified in different ways, such as by their target, chemical nature, physical state, and mode of action. Classification based on the target is perhaps the most widely known: insecticides, herbicides, fungicides, and rodenticides (Table 13.1).This chapter considers the chemistry, characteristics, and health effects of several representative groups of pesticides and herbicides. It then discusses several halogenated hydrocarbons that have become of much concern in recent years, including polychlorinated biphenyls (PCBs) and dioxins.

13.2 INSECTICIDES

13.2.1 Introduction

Insecticides are those compounds that are effective against insects. Many insecticides have been developed and used to control various species of insects. While most insecticides are applied as sprays, others are applied as dusts, aerosols, fumigants, and baits. The majority of insecticides used today are synthetic organic chemicals, and most of them are nerve poisons. They act by inhibiting the organism's enzymes or interacting with other target sites vital to

Table 13.1 Classification of Pesticides

Method of classification	Example
By target	Insecticides, herbicides, fungicides, rodenticides, algaecides, nematocides
By chemical nature	Natural organic compounds, inorganic compounds, chlorinated hydrocarbons, organophosphates, carbamates
By physical state	Dusts, dissolved solutions, suspended solutions, volatile solids
By mode of action	Contact poisons, fumigants, stomach poisons

the proper functioning of the insect's nervous system. Other insecticides act by blocking essential processes, such as respiration. Although there are many synthetic organic insecticides, this chapter focuses on three main groups: chlorinated hydrocarbons, organophosphorus compounds or organophosphates, and carbamates.

13.2.2 Chlorinated Hydrocarbons

13.2.2.1 Introduction

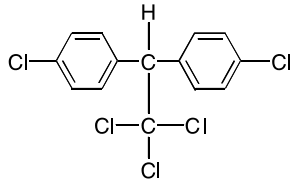
Chlorinated hydrocarbons, also called organochlorines, were the first commercial organic insecticides to be developed. DDT, aldrin, chlordane, dieldrin, endrin, lindane, and heptachlor are some examples (Figure 13.1).

13.2.2.2 DDT

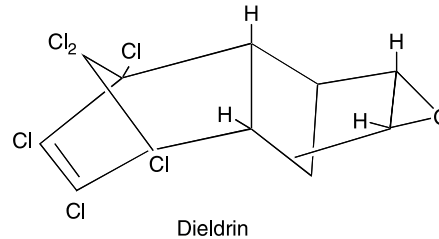
DDT (2,2-bis [p-chlorophenyl]-1,1,1-trichloroethane or dichloro-diphenyl trichloroethane), discovered as a pesticide in 1939, is probably the most widely known pesticide of the 20th century. It was first used for controlling disease-carrying insects, such as mosquitoes that spread malaria. As the range of DDT's effectiveness against insects became known, it was used by soldiers during World War II to control the body lice that spread typhus. After World War II, DDT was used in the home and applied to a variety of agricultural crops, providing enormous success in pest control. DDT proved effective in the control of a large number of pests, including gypsy moth, potato pests, corn earthworm, and codling moths. Because of DDT's impact on human disease control, the discoverer of DDT, Dr. Paul Müller, received the Nobel Prize in medicine in 1948. Despite these successes, some 20 years later, when DDT's environmental impacts became evident, its use was either limited or totally banned in industrialized countries, although it is still used in a number of less-developed countries.

DDT is characterized by its very low vapor pressure, extremely low solubility in water (1.2 ppb), and high solubility in oils. Because of this latter property, DDT can be readily absorbed through the skin into the fatty tissues of living organisms, and can biomagnify as it passes through the food chain. DDT is released slowly, when the stored fat is called upon as a source of energy. Of the two isomers of DDT, the *p,p'*-isomer is more toxic to invertebrates than the *o,p*-isomer.

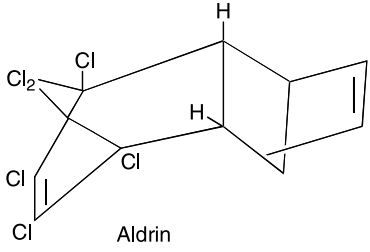
Typically, DDT and other chlorinated hydrocarbons are persistent broad-spectrum insecticides. Their residues persist in the environment for long periods, ranging from a few months to years. The half-life of DDT is estimated to be 7 to 30 years, depending on the environment. The organochlorines have broad-spectrum characteristics, enabling them to affect many different species of insects. Environmental persistence of this group of chemicals is due to the fact that they are not readily degraded by the action of water, heat, sunlight, or microorganisms. DDT rapidly accumulates in invertebrates, to several



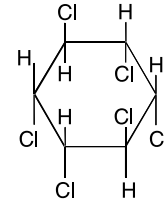
p, p′-DDT (Dichloro-diphenyl trichloroethane)



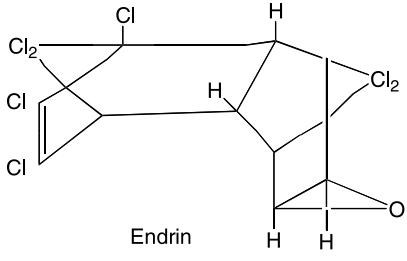
Dieldrin



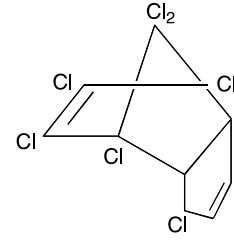
Aldrin



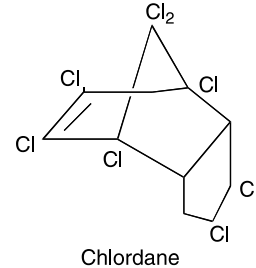
Lindane (1, 2, 3, 4, 5, 6-hexachlorocychlohexane)



Endrin



Heptachlor



Chlordane

Figure 13.1 Chemical structures of chlorinated hydrocarbon insecticides.

thousand times the exposure level in extremely low concentrations. The 96-hour LC_{50} for 19 species of fish ranges from 1.8 to 22 μg/l (Table 13.2). A 60% reproductive impairment was observed in *Daphnia* at 100 μg/l.

DDT adversely affects several physiological characteristics, including normal ratios of serum amino acids, thyroid activity, and the ability to withstand stress. Although DDT has not been shown to influence gonad

Table 13.2 Summary of Acute Toxicity of DDT for Fish

Test organism	Stage or wt (g)	96-hour LC_{50} (μg/l)
Black bullhead	1.2	4.8
Bluegill	1.5	8.6
Channel catfish	1.5	21.5
Coho salmon	1.0	4.0
Fathead minnow	1.2	12.2
Largemouth bass	0.8	1.5
Northern pike	0.7	2.7
Rainbow trout	1.0	8.7
Walleye	1.4	2.9
Yellow perch	1.4	9.0

maturation, the mortality of fry produced by DDT-treated parents is high, especially during the terminal stages of yolk absorption.[1]

DDT and other chlorinated hydrocarbons are very resistant to metabolic breakdown. Nevertheless, in animals and humans, DDT is degraded to DDE (ethylene 1,1-dichloro-2,2-bis(p-chlorophenyl) or dichlorodiphenyl dichloroethylene) or DDD (ethane 1,1-dichloro-2,2-bis(p-chlorophenyl)) (Figure 13.2). A limited conversion of DDT to DDE occurs in humans. The conversion is catalyzed by DDT dehydrogenase, and the resultant DDE is a stable metabolite.

Research conducted by Redetszke and Applegate[2] further demonstrated the persistence and biomagnification of chlorinated hydrocarbons. These researchers studied the residues of organochlorine pesticide in adipose tissue samples of 25 persons (19 males and 6 females) from El Paso, Texas. None of the tissue was taken from people known to have occupational exposure to pesticides. Eight organochlorine compounds were observed in the tissue samples. The pesticide residue levels were in the moderate range. DDE was found in all the samples tested, with an average level of 4.96 ppm, whereas the

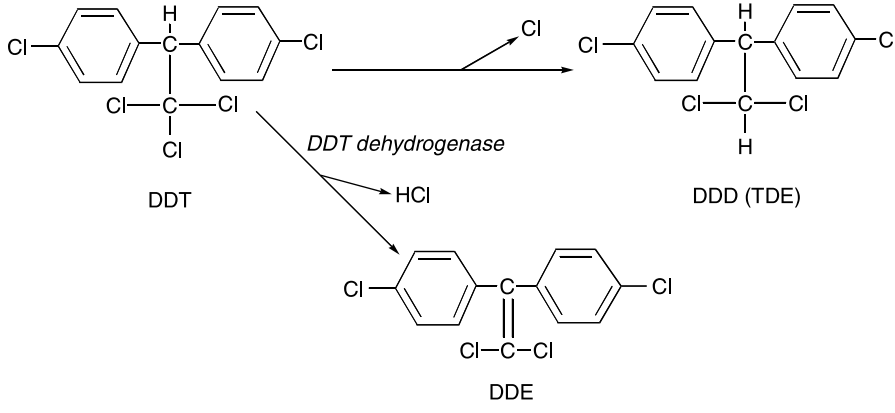


Figure 13.2 Metabolism of DDT.

average level of DDT was 1.50 ppm. Since DDE is a stable breakdown product of DDT (Figure 13.2), its presence in the tissue represents mainly past ingestion. It could also represent low-level indirect exposure from food and water from areas where DDT was used in the past and persists in the environment.

Nakata et al.[3] studied the levels of persistent organochlorines, such as DDTs, hexachlorocyclohexanes (HCHs), chlordane compounds (HCLs), and hexachlorobenzene (HCB), in a wide variety of foodstuffs and human tissues collected from Shanghai and its vicinity in China between 2000 and 2001. Among the organochlorine compounds analyzed, DDT and its metabolites were found to be prominent in most of the foodstuffs. In particular, mussels were found to contain 34 ppb (on lipid weight) of DDTs, levels that were one to three orders of magnitude greater than those reported in bivalves from other Asian countries. The levels of the other compounds in foodstuffs were found to be generally low, suggesting relatively small inputs into the environment. However, the researchers found high concentrations of DDTs and HCHs in human tissues from Shanghai, with the maximum values of 19 ppb and 17 ppb (lipid weight), respectively. The researchers concluded that, because foodstuffs are a main source of human exposure to contaminants, the greater concentration of DDTs and HCHs in the Chinese residents under study might be due to extensive uses of these compounds as agricultural pesticides in the past.

One of the most important health effects of DDT, DDE, and a number of other chlorinated hydrocarbons is on the endocrine system. Many studies have provided evidence suggesting that chlorinated hydrocarbon residues found in the environment may be responsible for interference with the functioning of the endocrine system and disruption of reproduction. Published reports relate observations of such disruption involving alligators in Lake Apopka, Florida, sea gulls in Tacoma and bald eagles on the Columbia River (both in the state of Washington), and trout in the U.K., among others. Louis Guillette, a zoologist, was credited with the initial observation that many of the Lake Apopka alligators exhibited abnormal reproductive systems and meager male hormones, apparently due to pesticide residues.[4] Field and laboratory studies have shown similar effects of a number of toxicants on wildlife. Observed effects include:

- feminization of male alligators and trout when exposed to hormone-like chemicals in laboratories
- poor reproduction among bald eagles along the Columbia River (seemingly linked to exposure to DDE and PCBs – see later section)
- offspring of exposed pregnant females showing: elevated testicular cancer and delayed puberty (in mice), malformed sex organs (in rats), and reduced sperm counts (in hamsters)
- salmon in the Great Lakes with enlarged thyroids and males with premature sexual development

Some scientists suggest that exposure to these chemicals could be related to the surge of disorders in human reproductive organs – from falling sperm counts to increasing rates of breast and prostate cancers – in the industrialized world since World War II (Chapter 14 deals with endocrine disrupters in depth.)

The adverse effects of organochlorine compounds on birds have been widely known since the publication of Rachel Carson's book *Silent Spring*. Not all species of birds have suffered equally, however. Birds of prey are especially susceptible to the persistent organochlorine insecticides, and the levels that inhibit reproduction can be very much lower than those that kill. For example, common species used in the laboratory, such as chicken, pheasant, pigeon or sparrow, can cope with insecticides far more successfully than other species. Birds that migrate lay down large amounts of fat prior to migration to serve as a store of energy. Because many pesticides are soluble in fat, birds accumulate the poison in their fat before migrating. The poison is then released to do its damage when fat is consumed during the journey.

Delegates from about 110 countries met in Geneva in September 1999 to work on a treaty to control 12 persistent organic pollutants [POPs]. They agreed to the international phase-out of the pesticides aldrin, endrin, and toxaphene. They also decided to severely restrict the use of four others – chlordane, dieldrin, heptachlor, and mirex – and one industrial chemical, hexachlorobenzene, allowing only some residual uses. These countries are aiming for a global treaty because these persistent bioaccumulative chemicals can be transported by wind and water and can cause damage to wildlife far from where they are originally used. These chemicals also are suspected of causing diseases of the immune system, reproductive disorders, and abnormal child development in humans, even at low doses. However, the countries were unable to make decisions on DDT, PCBs, dioxins, and furans. The World Health Organization (WHO), public health specialists, and some developing countries wanted DDT kept available for malaria control until equally inexpensive alternatives are developed.[4]

13.2.3 Organophosphorus Compounds

13.2.3.1 Introduction

Organophosphorus insecticides are the most toxic among the insecticides; they are dangerous not only to insects but also to mammals. Many of these compounds, such as parathion, paraoxon, timet, and tetram, are in the "super toxic" category of human poisons. Human fatal doses for these toxicants are < 5 mg/kg, along with arsenic (As), cyanide (CN^-) and some others. As little as 2 mg of parathion has been known to kill children. Figure 13.3a shows the chemical structure of three representative organophosphorus insecticides: parathion, malathion, and tetraethyl pyrophosphate (TEPP). Figure 13.3b shows several organophosphorus compounds or organophosphates: diisopropylphosphofluoridate (DIPF), sarin and tabun. These are highly toxic but are

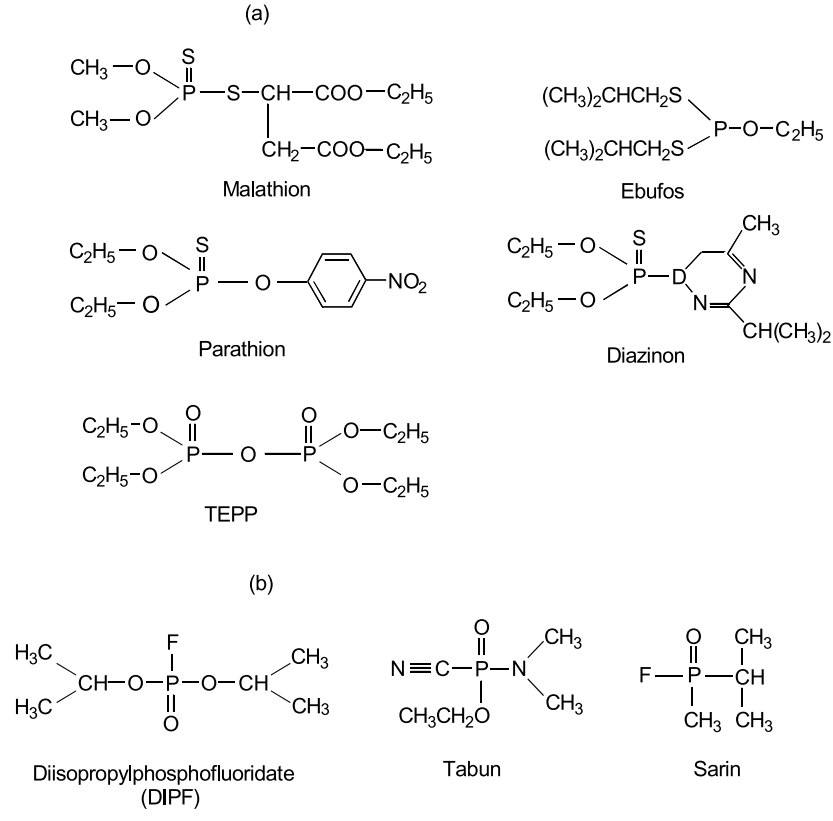


Figure 13.3 Chemical structures of organophosphate insecticides (a) and nerve gases (b).

not used as pesticides. Sarin and tabun are nerve gases used in chemical warfare. Diisopropylphosphofluoridate was initially intended for use in chemical warfare but was excluded because of its relatively lower toxicity compared with the other two agents.

13.2.3.2 Toxicity of Organophosphorus Compounds

Organophosphate insecticides are very toxic and exposure-related health problems have been encountered, especially in the earlier days of application. Symptoms of poisoning in humans include nausea, vomiting, diarrhea, cramps, sweating, salivation, blurred vision, and muscular tremors. Severe cases may be fatal due to respiratory failure. Even though organophosphates are usually more toxic to humans and mammals than chlorinated hydrocarbons, they are more easily biodegraded than the organochlorines. Because they do not persist in the environment or accumulate in fatty tissue, they have virtually replaced the organochlorines for most uses.[5]

13.2.3.3 Action of Acetylcholinesterase and Organophosphates

The mode of action of organophosphorus insecticides in vertebrates and invertebrates is the inhibition of *acetylcholinesterase* (AChE), the enzyme responsible for the breakdown of the neurotransmitter acetylcholine (ACh). Acetylcholine, in turn, is produced from choline and acetyl CoA by choline acetyltransferase (Reaction 13.1 and Reaction 13.2). Inhibition of the enzyme results in accumulation of ACh at the nerve endings, leading to disruption of nervous activity. As shown in the reactions, subsequent to breakdown by AChE, ACh is regenerated from choline. The resultant acetic acid from Reaction 13.1 is activated to acetyl CoA before reacting with choline.

$$(CH_3)_3N^+-CH_2CH_2-O-\overset{\overset{O}{\|}}{C}-CH_3 \xrightarrow{AChE} (CH_3)_3N^+-CH_2CH_2OH + CH_3COOH$$

Acetylcholine (ACh) Choline Acetic acid

(13.1)

$$\text{Choline + acetyl CoA} \xrightarrow{\textit{Cholineacetyl transferase}} \text{Acetylcholine} \qquad (13.2)$$

Because of the important role that AChE plays, it is worthwhile reviewing the principles of nerve transmission. The junctions between adjacent neurons are termed *synapses* (Figure 13.4). Nerve impulses, also called *action potentials*, are transient changes in the membrane potential that move rapidly along nerve cells. Action potentials are created when the membrane is locally depolarized by about 20 mV. This small change is sufficient to dramatically influence the

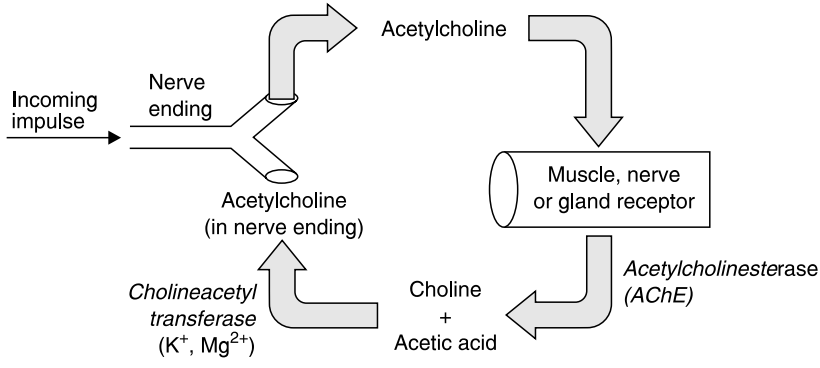


Figure 13.4 Action of acetylcholine and acetylcholinesterase at a synapse.

specific proteins in the axon membrane, called *voltage-gated ion channels*. These proteins are ion channels that are specific either for sodium ions (Na^+) or potassium ions (K^+). The ion channels are normally closed at the resting potential of −60 mV. When the potential difference rises to −40 mV, the "gates" of the Na^+ channels will be opened, causing Na^+ ions to flow into the cell. The membrane potential continues to increase after the entrance of Na^+ ions, opening additional Na^+ channels. In this way, the action potential moves down the axon in a wave-like manner. The potential rises to more than +30 mV, then the influx slows and stops. As the Na^+ channels close, K^+ channels begin to open and K^+ ions rush out of the cell, returning the membrane potential to the negative value. The potential eventually overshoots its resting value, when K^+ channels close. The resting potential is eventually restored by the action of the Na^+,K^+-ATPase and the other channels.[6]

The cell-to-cell communication at the synapse is mediated by ACh. A brief summary of this system of communication is given below:

1. The arrival of an action potential at the synaptic knob opens Ca^{2+} channels in the presynaptic membrane.
2. Influx of Ca^{2+} induces the fusion of ACh-containing vesicles with the plasma membrane and release of ACh into the synaptic cleft.
3. Binding of ACh to receptors in the postsynaptic membrane opens Na^+ channels.
4. The influx of Na^+ depolarizes the postsynaptic membrane, generating a new action potential.

AChE has a reactive serine at the active site that is a vulnerable target for organophosphate inhibitors. Inhibition of the enzyme results in accumulation of ACh at the nerve endings, causing disruption to synaptic activity. Evidence indicates that the vertebrate AChE contains two binding sites, and it is likely that the insect enzyme is similar. The anionic site, which may contain a glutamate residue, interacts with the positively charged nitrogen (N) atom of ACh, while the esteratic site is responsible for the cleavage of the ester link of ACh. The esteratic site contains a serine residue, whose nucleophilicity is enhanced by hydrogen bonding to the imidazole group of a neighboring histidine residue. Chemicals such as organophosphate insecticides that can inactivate AChE are known to attach to the $–CH_2OH$ residue of the esteratic site of the enzyme by forming a covalent bond. They are therefore often called covalent inhibitors of AChE.

13.2.4 Carbamates

In the same way that organophosphate insecticides, such as parathion and malathion, are derivatives of phosphoric acid, the carbamates are derivatives of carbamic acid ($HO–CO–NH_2$). Carbamates are widely used for worm control on vegetables. Examples of carbamates include aldicarb (2-methyl-2-[methylthio]propionaldehyde-*O*-[methylcarbamoyl] oxime) (Figure 13.5) and

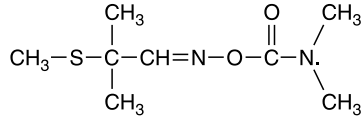


Figure 13.5 Chemical structure of aldicarb.

carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate). The mode of action of the carbamates is the same as that of organophosphates, i.e., inhibition of AChE.

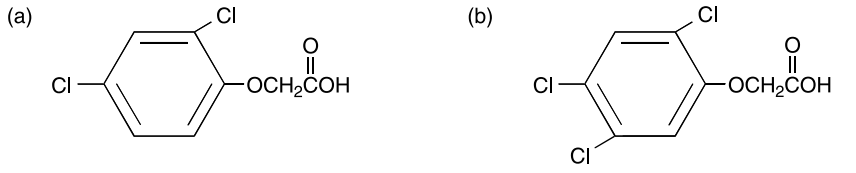
Aldicarb (trade name Temik) is one of the most widely used carbamates. The first time it was detected in groundwater was in Suffolk County, New York, in August 1979. Although laboratory and field studies indicated that the pesticide could not reach groundwater, a combination of circumstances led the residues to reach groundwater and to be ingested by humans. A monitoring program revealed that 1121 (13.5%) of 8404 wells tested exceeded the state's recommended guideline of 7 ppb. Of the contaminated wells, 52% contained 8 to 30 ppb aldicarb, 32% contained 31 to 75 ppb, and 16% more than 75 ppb. Studies did not, however, reveal any cases of carbamate poisoning.[7]

Case Study 13.1

Another aldicarb episode occurred in four western states (California, Washington, Oregon, and Alaska) and one Canadian province (British Columbia) in 1986. About 300 people were made ill over the long July 4 weekend after eating watermelons contaminated with aldicarb. The melons were grown on farms in southern California. Forty of 550 watermelon fields in California were shown to be contaminated with the pesticide. As a result, about one million melons were destroyed. Aldicarb is manufactured by Union Carbide. Its approved use is on a number of crops to control nematodes, aphids, and other insects that feed on parts of crop plants. It is not approved for use on watermelons. It was reported that a concentration of aldicarb of 0.2 ppm in watermelon fruit caused illness. The contaminated melons had concentrations up to 3 ppm. Symptoms resembled those of influenza, i.e., blurred vision, perspiration, nausea, dizziness, and shaking. These symptoms usually disappear after a few hours. In this episode, none of the cases proved fatal.

13.3 HERBICIDES

During the Vietnam War, the U.S. Air Force's defoliation program applied a huge quantity of undiluted 2,4-D (2,4-dichlorophenoxy acetic acid) and 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) (Figure 13.6) on Vietnam's agricultural and forest land between 1965 and 1970. In addition to military use in Vietnam, phenoxyherbicides (PHs) were widely used in the U.S. for controlling weeds in agriculture and rangeland, lakes and ponds, and in forests.



2, 4-D (2, 4-dichlorophenoxy) acetic acid

2, 4, 5-T (2, 4, 5-trichlorophenoxy) acetic acid

FIGURE 13.6 Chemical structures of (a) 2,4-D, and (b) 2,4,5-T.

As shown in Figures 13.6, 2,4-D and 2,4,5-T are identical esters, except for the additional chlorine (Cl) atom present on the benzene ring of 2,4,5-T. During production of these two compounds, chlorinated dioxins (TCDD) (to be discussed in Section 13.6) were found to contaminate the final product, a compounding factor in analysis because of its high toxicity. Prior to its ban in 1978, 2,4,5-T was used in combination with other chemicals in forestry, primarily for "releasing" conifer species from competition with broadleaf species. PHs are also used after logging to clear the brush so that seedlings can be planted.

The biochemical actions of PHs in plants are complex. After application, the chemicals are absorbed primarily through stomata and secondarily through root hairs with water. In resistant species, PHs are detoxified by various decarboxylation and conjugation reactions. In sensitive plants, the chemicals disrupt growth and various metabolic processes as they are translocated through vascular tissue. Growth and metabolic processes are affected by the stimulation or inhibition of many enzymes, possibly leading to plant death. Certain species, such as Douglas fir, are tolerant when PHs are mixed with a water carrier.

Numerous clinical reports in humans have described peripheral neuropathy (degeneration of nervous tissue) and acute myopathy (disorder of muscle tissue or muscles) after dermal exposure or oral ingestion of 2,4-D. Clinical symptoms of severely poisoned farmers include pain and weakness in the lower extremities, slowed nerve conduction velocity, twitching, and muscle spasms. In addition, behavioral changes, such as nervousness, inability to concentrate, irritability, impotence, and others, may occur.[8] These symptoms have also been found in studies involving workers employed at PH manufacturing plants. In the early studies, the degree of TCDD contamination was often unknown. In later studies, exposure is primarily to the formulated product.

The neurotoxic and mycotoxic mechanisms of 2,4-D are not well studied.[9] In recent years, several investigations have been made involving nerve conduction velocity (NCV) measurement. This approach has become increasingly valuable in xenobiotic assessment because slowed NCV is associated with histological as well as behavioral changes. NCV is an excellent starting point for epidemiology because the techniques involved are rapid, accurate, and noninvasive. In 1979, a survey was conducted of 190 current, former, and

retired workers of a plant in Jacksonville, Arkansas, where PHs had been produced for 20 years.[10] Workers and control subjects were carefully screened in order to minimize factors that could possibly affect NCV. Three nerves were tested (median motor, median sensory, and sural), measured, and recorded for 56 workers at the plant. The results showed that 46% of the study group had one or more slowed NCVs. In addition, slowed sural NCV was correlated to duration of employment at the factory.[10]

The widespread use of PHs during the Vietnam War has been associated with a large variety of health problems. Again, TCDD is a complexing factor. Specific neurotoxic effects of 2,4-D have recently been examined in response to reports of episodic increase in intracranial skull pressure associated with insecticide intoxication.[11] These symptoms prompted the first research involving central neural metabolism of 2,4-D, specifically concerning the accumulation and transport within the brain and spinal cord.

PHs were banned for forestry in 1979 due to a combination of public pressure and the results of the U.S. Environmental Protection Agency (EPA)'s *Alsea II* report. This widely criticized report found significantly greater spontaneous abortion rates inside a residential area exposed to PH spray when compared with a similar area without spray. Although banned for use in forestry, PHs are still widely used as herbicides for cotton, corn, wheat, and rice crops.

13.4 POLYCHLORINATED BIPHENYLS

13.4.1 Introduction

Polychlorinated biphenyls (PCBs) are a class of synthetic chlorinated organic compounds with biphenyl as the basic structural unit. Chlorination of the basic structure can theoretically yield 209 chlorobiphenyls substituted with 1 to 10 chlorine atoms, but the probable number of compounds is estimated to be 102. The general chemical structure of PCBs is shown in Figure 13.7.

Although PCBs are chlorinated hydrocarbons, they are not pesticides. However, because of their wide use and resistance to degradation in the environment, PCBs are known as one of the major organochlorine pollutants found in the environment. Extensive PCB-contamination exists in the food chain throughout the world.

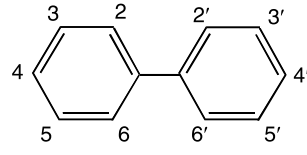


Figure 13.7 Chemical structure of PCBs (numbers are possible sites for Cl).

13.4.2 Properties of PCBs

The properties of PCBs are similar to those of DDT. PCBs are soluble in fat or fat-solvents, but are hardly soluble in water. The solubility of PCBs in water and in organic solvents affects their transport and persistence in the environment. Their solubility in water generally decreases with increase in the degree of chlorination. Individual chlorobiphenyls vary in their solubility, from about 6 ppm for monochlorinated biphenyls to as low as 0.07 ppm for octachlorobiphenyls.[12] They are non-drying, and non-flammable (they are stable on long heating at 150°C), do not support combustion when alone above 360°C, and can withstand temperatures up to 650°C (1600°F). They are not affected by boiling with NaOH solutions. Electrically, PCBs are nonconducting. PCBs also have very low vapor pressures, which, like their solubility in water, decrease with increased chlorination.

PCBs tend to bind tightly to particulate matter, such as soils and sediments. Therefore, surface waters with low particulate loads may have very low concentrations of PCBs, while high concentrations may exist in bottom sediments.

13.4.3 Uses of PCBs

PCBs were first manufactured commercially in 1929 in the U.S. by the Monsanto Chemical Company, using the trade name of Aroclor followed by serial numbers (such as 1221, 1248, and 1268, etc.). The last two digits in the serial numbers refer to the percentage of chlorine in the products. This nomenclature has recently been replaced by the International Union of Pure and Applied Chemistry (IUPAC) PCB nomenclature. Appendix 2 presents a summary of the nomenclature for this group of compounds.

Because of their unique properties, PCBs were widely used. Industrial uses include manufacture of plastics, paints, varnishes, asphalt, rubber, carbon paper, carbonless paper, printing inks, synthetic adhesives, sealers in waterproof material, lubricating oils, fire retardants, electrical transformers, and capacitors in the power industry.[13] Although PCBs are not pesticides, they were previously added to DDT to extend its "kill effect."

The U.S. banned the use of PCBs in 1976 in the wake of concern about public health. In 1985, the EPA issued a final rule requiring removal of PCB fluids, or electrical transformers containing PCBs, from commercial buildings by October 1, 1990.

13.4.4 Environmental Contamination by PCBs

Like DDT, PCBs are ubiquitous in the environment. Contamination by PCBs may occur through various activities, including:

- spills and losses in manufacture of PCBs and PCB-containing fluids
- vaporization or leaching from PCB formulations

- leaks from sealed transformers and heat exchangers
- leaks of PCB-containing fluids from hydraulic systems that are only partially sealed
- disposal of waste PCBs or PCB-containing fluids[14]

In addition, PCBs are released into the air or waterways by the incineration of rubber and plastics, and through the use of pesticides that contain added PCBs.

One of the most important routes by which PCBs can contaminate the environment is air. Airborne PCBs can rapidly and efficiently dissipate from point sources to distant areas. In addition to the airborne route, marine environments receive PCBs from various sources, including rivers, urban runoff, wastewater discharges, and dumped sewage sludge. Like DDT, once in the aquatic environment PCBs tend to bioaccumulate. PCBs and DDT are similar to each other in terms of their low water solubilities, extreme lipophilicity, and great resistance to degradation.[15]

13.4.4.1 Wildlife Exposure to PCBs

PCBs were identified in birds' feathers as early as 1944, and many investigators have since reported varying levels in wildlife in Canada, Germany, Great Britain, Japan, the Netherlands, Sweden, and the U.S. High concentrations of the compounds have been found in fish taken from the Great Lakes,[16] the Hudson River, and Tokyo Bay. Polar bears and fish in the Arctic tundra lakes also contain PCB residues, as do birds living in Antarctic waters.

The presence of PCBs in the Great Lakes is still of considerable concern, even though the use and manufacture of PCBs were banned in the 1970s. Concerning the risk to the Great Lakes system, a new index based on fate, persistence, and toxicity ranked PCBs second to dioxins. The primary concern for the public is the danger of PCBs present in consumable fish. Studies carried out by the Wisconsin Department of Natural Resources on coho and chinook salmon in Lake Michigan showed general decreases in the PCB levels between 1974 and 1990. For example, the highest sample mean for coho PCBs was found in fish samples obtained in 1976, with a value of 14.25 mg/kg, while the highest sample mean for chinook PCBs occurred in 1974, with a value of 11.69 mg/kg. Sample means in 1990 decreased to 0.83 and 1.17 mg/kg for coho and chinook, respectively.[17]

However, PCB concentrations in fish are related to a number of factors, such as the size and fat content of the fish, and the food web structure. Furthermore, slower-growing fish can accumulate higher levels of contaminants than faster-growing fish. This is because faster-growing fish gain more body mass for each unit mass of contaminant they consume than do slower growing fish.

The decreases in PCB concentrations mentioned above appear to be diminishing, and there is concern that a slow increase in PCB concentrations in the fish is now occurring. Although the reasons for this change are not well

known, some researchers suggest that the increase may be related to the decline in the alewife population in Lake Michigan that began in early 1980s. Since alewife is an important food source for both coho and chinook salmon, it is suspected that the decline in alewife has led to slowed growth in coho and chinook, leading to increased levels of PCBs.[17]

Otto and Moon[18] collected brown bullheads (*Ameiurus nebulosus*) from the St. Lawrence River and compared their detoxification capacities to bullheads from a relatively nonpolluted aquatic system, Lac La Peche in Canada. They observed that the content of PCBs in white muscle was significantly higher (22-fold) in bullheads from the St. Lawrence River compared with those from Lac La Peche. Activities of liver ethoxyresorufin O-deethylase (EROD) were 2.8-fold higher in St. Lawrence River bullheads than in fish from Lac La Peche. (As noted previously, EROD is widely used as a biomarker for pollution by synthetic organic compounds, particularly chlorinated hydrocarbons.)

13.4.4.2 Human Exposure to PCBs

Human exposure to PCBs is the combined result of intake from air, water, and food sources, the majority being attributable to consumption of fish (except for sporadic instances of contamination). Exposure through inhalation is not likely to exceed 1 mg/day and the amount taken in drinking water is at most 5 to 10 mg/day.[19] Thus, even in highly industrialized areas, these represent minor sources of PCB intake. According to FDA market basket surveys during the 1970s, the average adult in the U.S. received 5 to 10 mg PCB/day in the diet.[20] The value fluctuates widely because PCBs are found primarily in meat, poultry, and, especially, fish products. Individuals who eat large quantities of fish or who eat fish from polluted areas two or three decades ago would have intakes in excess of 100 mg/day.

The most highly documented case of PCB poisoning in humans is known as yu-sho or "oil disease," which occurred in southwest Japan in 1968. The disease was caused by ingestion of rice oil contaminated by a commercial brand of Japanese PCB, Kanechlor 400. This particular PCB brand contained 48% chlorine and was found in the contaminated rice oil at concentrations from 2000 to 3000 ppm.[21] By the end of 1982, more than 1700 persons were identified as having been poisoned.

Another highly documented case of PCB poisoning was that called yu-cheng (the Chinese for "oil disease"), which occurred in central Taiwan in 1979. Again, contaminated cooking oil was the source. By the beginning of 1983, 2060 persons had been identified as victims. The total average intake of PCBs by the victims of yu-sho and yu-cheng was estimated to be 633 mg and 973 mg, respectively.[22]

13.4.5 Metabolism of PCBs

In humans and animals, PCBs are absorbed from the gastrointestinal tract and distributed rapidly to all tissues. Elimination of the absorbed PCBs from the

body occurs slowly, with its extent being dependent upon the number of chlorine atoms on the PCB molecule. Like other polycyclic aromatic hydrocarbons, PCBs are metabolized by the microsomal mixed-function oxidase (MFO) system. Through hydroxylation and conjugation with glucuronic acid, the polarity of the PCB molecules is enhanced, thereby increasing their solubility in body fluids and allowing excretion.[23] This process is strongly dependent on the location and degree of chlorination of the biphenyl molecule. The rate of metabolism and excretion decreases as the number of chlorines increases. Therefore, mono-chlorobiphenyls are metabolized and excreted faster than di-chlorobiphenyls, which are processed faster than tetrachlorobiphenyls. The degree of chlorination also affects how PCBs are eliminated from the body: mono- and di-chlorobiphenyls are largely excreted in the urine, whereas PCBs with higher numbers of chlorine atoms are excreted primarily in the feces.[23]

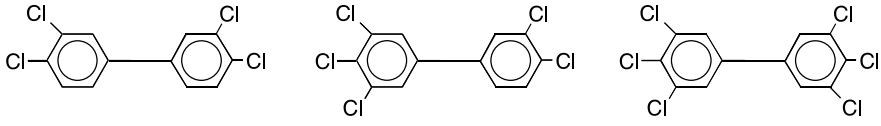
When the number of chlorine atoms on the biphenyl molecule is four or more, the position of the chlorine atoms becomes important in determining the rate of metabolism and excretion of the PCB species. The primary requirement for more rapid metabolism is the presence of two adjacent unsubstituted carbon atoms on the biphenyl molecule.

Like DDT and its metabolites, PCBs stored in adipose tissue are mobilized into the liver under starvation stress. Because PCBs are metabolized in the liver, the health of the liver is critical. When the liver cells are damaged by certain drugs or toxicants, such as CCl_4 for example, the liver will not be able to perform its detoxification process effectively.

A possible route of environmental breakdown of PCBs is through photolysis or photochemical process. PCBs absorb ultraviolet (UV) radiation in the 200 to 300 nm range, leading to dechlorination. This causes PCBs to be converted to a less harmful state, the biphenyl product. Several factors influence the photolysis, notably the degree of chlorination, position of Cl substitution in the ring, and environmental factors. Although photolysis of certain PCB analogs has been demonstrated experimentally, the extent to which the reaction occurs in the environment is less known. Environmental degradation of PCBs also occurs in soils, lakes, rivers, and sediments, by the activities of both aerobic and anaerobic microorganisms. As a result, less-chlorinated chlorobiphenyls are produced. The main mechanism involved in the biodegradation is hydroxylation, while ring cleavage may also occur.

13.4.6 Toxicity of PCBs

Studies indicate that the toxicity of technical PCB mixtures may be due to the presence of trace levels of several PCB congeners with four or more Cl atoms at both *para* and *meta* positions in the biphenyl rings but no Cl atoms in *ortho* positions.[24] Among the 20 possible coplanar PCB congeners 3,3′,4,4′-tetrachlorobiphenyl, 3,3′,4,4′,5-pentachlorobiphenyl and 3,3′,4,4′, 5,5′-hexa-chlorobipheynyl (Figure 13.8) were found to be the most toxic. These three coplanar congeners and dioxin were considered responsible for eliciting toxic



3, 3′, 4, 4′-Tetrachloro-biphenyl | 3, 3′, 4, 4′, 5-Pentachloro-biphenyl | 3, 3′, 4, 4′, 5, 5′-Hexachloro-biphenyl

Figure 13.8 Chemical structures of three coplanar PCB congeners.

effects in laboratory animals, including body weight loss, dermal disorder, hepatic damage, thymic atrophy, teratogenicity, reproductive toxicity, and immunotoxicity.[24]

The symptoms reported in both yu-sho and yu-cheng episodes included increased whitish eye discharge and swelling of the upper eyelids, pigmentation of nails, skin and mucous membranes, acne-like skin eruption (chloracne) with secondary infections, feelings of weakness, headache, and vomiting. Three to four years after both incidences, the skin of those people who were only mildly poisoned appeared normal, yet systematic disorders, including dullness, cough, headache, stomachache, and swelling and pain of the joints, persisted.[25] By 1984, 24 of the people poisoned in Taiwan had died of liver cirrhosis or hepatomas. Additionally, 39 babies born to women who had been poisoned suffered from hyperpigmentation, and eight of them died soon after birth. Those children who did survive showed obvious signs of growth retardation. Of the yu-sho victims, 112 people had died by the end of 1982. However, the causes of only 31 deaths were confirmed, 11 were from neoplasms, primarily of the stomach, liver, and lung.[22] Other clinical manifestations of PCB poisoning include dental, endocrine, neurological, and hematological disorders.

13.4.7 Biological Effects of PCBs

Studies have shown that PCB poisoning leads to metabolic changes in human victims. These changes may be caused primarily by the dysfunction of metabolic organs, and secondarily by accelerated metabolism through enzyme induction. For example, exposure to PCBs causes an altered general lipid metabolism. An elevated concentration of serum triglyceride was commonly observed among victims of PCB poisoning. Since a significant positive correlation occurred between the triglyceride concentration and the blood PCB concentration, it is suggested that PCBs may be responsible for the hypertriglyceridemia. The hypertriglyceridemia appears to be due to disturbance of plasma triglyceride removal caused by diminished lipoprotein lipase following PCB exposure.[26]

PCBs, like other chlorinated hydrocarbons, exhibit high binding affinity to hepatic cytosolic receptor protein (*Ah* receptor) and induction potency of hepatic microsomal enzymes.[24] An increase in hepatic microsomal enzymes may result in an increased metabolism of endogenous substances, including

some hormones. For instance, PCBs have been reported to cause an increased degradation of estradiol, as evidenced by the lowered serum levels of the hormone among the Japanese female victims of PCB poisoning.

PCBs cause heme depletion by inhibiting uroporphyrinogen decarboxylase, an enzyme involved in heme synthesis (see Section 12.2.4). Because such depletion has a negative feedback effect, it increases the synthesis of ALA synthetase, which ultimately leads to uroporphyrin accumulation in the liver. PCBs also influence the metabolism of vitamin A. In animal experiments, rats fed diets containing 20 ppm PCBs showed a decreased storage of vitamin A. Suggested mechanisms for the decline include PCB-induced reduction of serum retinol-binding-protein and increases in microsomal enzymes that metabolize vitamin A.

13.5 POLYBROMINATED BIPHENYLS

13.5.1 Introduction

Polybrominated biphenyls (PBBs) are another group of halogenated aromatic hydrocarbons. PBBs were used predominantly as flame-retardants in thermoplastics, and about 5000 t of the material were manufactured in the U.S. between 1970 and 1975.

Between May and June 1973, a chemical company in Michigan mistakenly sent 227 to 454 kg (500 to 1000 lb) of PBBs to a grain elevator in south Michigan in place of magnesium oxide, a livestock feed additive. Subsequently, the PBBs were mixed into feed for cattle and other farm animals, which were then slaughtered and sent to market, ultimately contaminating a majority of the state's population. The contamination necessitated slaughter of more than 35,000 head of cattle, 1.6 million chickens, and thousands of pigs on 1000 Michigan farms. The total damage cost was $500 million. Since Michigan is a meat-, milk-, and egg-deficit state, the contamination was, for the most part, limited to Michigan. Because of this event, PBBs are no longer manufactured in the U.S.

13.5.2 Chemistry of PBBs

There are numerous isomers of PBBs, but commercial products usually have one to six bromine atoms. The chemical structures of several representative PBB isomers are shown in Figure 13.9. PBBs are lipophilic, poorly metabolized, and slowly excreted. The metabolites are hydroxyl derivatives. When a dose of monobromobiphenyl was injected into rabbits, 1% of the compound was found as a hydroxylated metabolite.

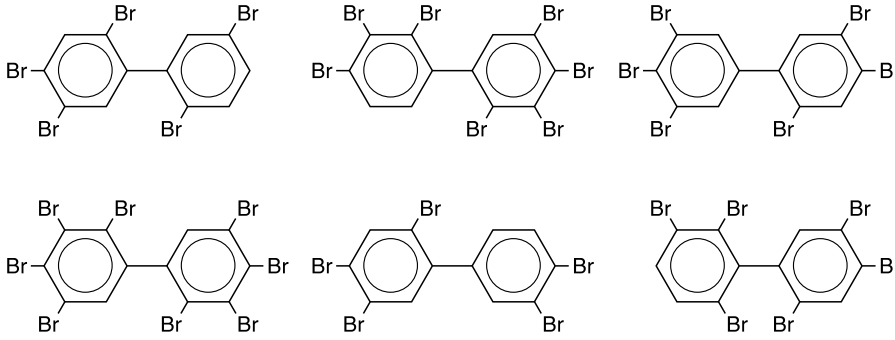


Figure 13.9 Chemical structures of several PBB isomers.

13.5.3 Toxicity of PBBs

PBBs are extremely persistent. When ingested, they remain in the body fat, perhaps indefinitely. They are toxic to the skin, kidneys, testicles, and adrenal gland, and cause liver damage, including liver tumors, and birth defects. In cows, milk production is decreased, coats become rough, and hoof deformities occur.

In humans, the ailments vary between individuals. Observable symptoms include nervousness, sleepiness, weakness, fatigue, lethargy, severe headaches, memory loss, nausea, joint swelling, and pain in the back and legs. Disorders in the skin, such as dryness, and nail discoloration, occur. Gastrointestinal problems are common. In a survey of 2000 individuals selected to be representatives of the population of Michigan, more than 90% had PBB concentrations of higher than 10 ppb in body fat, while members of the general population had no detectable levels. The FDA declared < 0.3 ppm as the safety level in meat and dairy products. If beyond that level, animals were to be quarantined by the state.

13.5.4 Biological Effects of PBBs

Like PCBs, PBBs are potent inducers of hepatic microsomal drug metabolizing enzymes. In the cell, PBBs act on mitochondria and disrupt energy production of all cellular processes. Clinical observations among the contaminated farmers in Michigan showed an elevated activity of serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), and lactic acid dehydrogenase (LDH). Immunological studies showed decreases in absolute number and percentage of T and B lymphocytes, and significant reduction of *in vitro* immune function. Interestingly, however, neither the subjective nor the objective findings correlated with either serum or fat PBB levels.

13.6 DIOXINS

13.6.1 Introduction

Dioxin refers to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and is a congener of the family of polychlorinated dibenzo-*p*-dioxins (PCDDs). PCDDs and polychlorinated dibenzofurans (PCDFs), unlike PCBs, have not been purposely manufactured. Rather, they are present as impurities associated with the synthesis of chlorophenols. PCDDs are one of the most toxic substances known and, like PCBs, are ubiquitous in the environment. There are 75 dibenzo-*p*-dioxins containing chlorine atoms. Figure 13.10 shows the general structure of PCDDs.

13.6.2 Exposure to Dioxins

Human exposure to PCDDs has been associated with workers engaged in the manufacture of technical chlorophenols and their derivatives, such as the herbicide 2,4,5-T.[27] The main sources of PCDDs in the environment include combustion-related processes, municipal-waste and medical-waste incinerators, pentachlorophenol formulations, numerous industrial manufacturing and chemical-formulation processes, fires, and urban runoff and stormwater.[28] The formation of PCDDs by pyrolysis of PCBs and chlorinated benzenes was observed in 1982 as the result of an electrical transformer fire in Birmingham, New York.

Humans are exposed to dioxin through herbicides in the air and soil, consumption of fish and meats, improper industrial-waste disposal (such as occurred in Times Beach, MO), and industrial accidents (such as the chemical plant accident in Seveso, Italy).[27] However, the most well-known human exposure to the chemical is the defoliant Agent Orange, used in the Vietnam War. Agent Orange, a combination of the herbicides 2,4-D and 2,4,5-T, was sprayed over the dense jungles of Vietnam to clear brush and trees that provided cover to the enemy. The herbicide was contaminated with small amounts (average 2 ppm) of TCDD. Agent Orange became the center of the health controversy after the war. During the 1970s, Vietnam veterans with a variety of illnesses began to blame their medical problems on Agent Orange exposure.

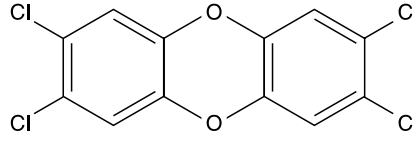


Figure 13.10 Chemical structure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

13.6.3 Toxicity of Dioxins

13.6.3.1 Toxicity of Dioxins in Animals

The acute toxicity of TCDD, expressed as LD_{50}, in a number of laboratory animals varies considerably with species. For example, the LD_{50} for guinea pigs is 0.6 mg/kg body weight, whereas the values for the mouse and hamster are 114 and 5000 mg/kg body weight, respectively. Perhaps one of the most significant characteristics of dioxin is that it has different effects on different species. Major symptoms exhibited in animals include:

- abnormal cell proliferations or organ enlargement, such as seen in lung, skin, gastric mucosa, intestinal mucosa, urinary tract, and bile duct and gall bladder
- atrophy or decreased cell proliferation in thymus, bone marrow, and testicle
- other effects such as liver lesions and edema[27]

In rodents, adverse effects on reproduction, immune function, lipid and glucose metabolism, and behavior have also been reported.[29] Guinea pigs exposed to dioxins exhibit loss of lymphoid tissue, particularly from thymus, thus becoming more susceptible to infections, although death does not result from infections. They die from a starvation-like wasting of the entire animal. Liver damage is less severe. Dioxins can inhibit sex hormones, and may induce adverse effects on insulin, increasing the chance of developing diabetes.[30] Chronic effects of dioxins in animals also vary with species. For example, PCDDs may be fetotoxic to some species (e.g., monkeys), but teratogenic to others, such as mice. However, dioxins' high toxicity to the mother means that the range in which dioxins cause toxic effects on the fetus but not on the mother is very narrow. Therefore, some toxicologists classify dioxins as a weak teratogen. Ironically, the fact that humans appear to be less sensitive to the acute effects of dioxins means that it could be a more potent teratogen for humans than it seems to be for laboratory animals.

The toxicity of dioxins varies widely from species to species, but the wasting away of tissue in exposed animals appears to be common to all animal species studied. As mentioned previously, tissue wastage is probably the cause of death in the very sensitive guinea pig. Dioxin exposure may, in addition, impair cell membrane proliferation.

Studies with animals suggest a strong connection between dioxin and endometriosis (the presence of uterine lining in other pelvic organs, especially the ovaries, characterized by cyst formation, adhesions, and menstrual pains). Scientists at the University of Wisconsin and others demonstrated that monkeys exposed to dioxins developed the disease, and that the incidence of the disease correlated with dioxin doses. For example, 71% of monkeys exposed to 25 ppt developed moderate to severe disease, while only 42% of animals fed 5 ppt developed the disease. By contrast, the control group of animals not fed dioxin had neither moderate nor severe disease.[31]

Studies on rats and mice show that dioxins are extremely potent carcinogens in these animals. Female rats fed varying doses of dioxins were shown to develop liver tumors. In addition, at high doses both male and female rats developed increased numbers of tumors in the mouth, nose, and lungs, as well as in the liver. It is suspected that dioxins may be about three times as potent a carcinogen as aflatoxin B_1, which is one of the most potent carcinogens known. In another study, scientists observed increases in thyroid tumors in male rats. Researchers consider that TCDDs may act as a promoter rather than initiator (see Chapter 16).

13.6.3.2 Toxicity of Dioxins in Birds

As is widely known, a series of dramatic avian population declines occurred in a number of countries during late 1940s and early 1950s. The declines were mainly associated with reproductive failure, characterized by marked thinning of eggshells, poor hatching, and lowered numbers of chicks surviving a couple of weeks. Most of these reproductive effects were correlated with exposure to xenobiotics, particularly DDT and dioxin-like compounds. Furthermore, the observed deformity or anatomical malformations were found to be associated with egg concentrations of PCDDs, PCDFs, and dioxin-like PCBs.

Many studies have since been conducted on the contaminations of birds by PCDDs and PCDFs. Wiesmuller et al.[32] measured the concentrations of PCDDs, PCDFs, and PCBs in unsuccessfully hatched eggs of three species of predatory birds – hobbies, goshawks, and sparrowhawks – collected in the Berlin-Brandenburg region of Germany. By use of toxic equivalency factors (TEQ) for birds, the researchers found that eggs of hobbies contained mean concentrations of 475 pg TEQ/g fat and 551 pg TEQ/g fat contributed by PCDD/PCDFs and coplanar PCBs, respectively. The researchers also found that, with the exception of one location, the burdens of TEQ originating from PCDD/PCDFs decreased steadily from 1991 until 1998.

A similar study was conducted on the concentrations of PCDDs, PCDFs, and non- and mono-*ortho*-chlorine-substituted biphenyls (dioxin-like PCBs) in livers of 17 species of birds collected in Japan.[33] The birds were grouped into granivores, piscivores, omnivores, and predators, based on their feeding habits. The researchers found the ranges of liver concentrations of PCDD/PCDFs by omnivores, piscivores, and predators to be 2300 to 8000 pg/g, 61 to 12,000 pg/g, and 480 to 490,000 pg/g on a fat weight basis, respectively. Livers of granivores contained relatively low concentrations of PCDD/PCDFs (80 to 660 pg/g). According to the authors, this is the first study on those toxicants in livers of several species of birds in Japan.[33]

13.6.3.3 Toxicity of Dioxins in Humans

The first studies of dioxins in people were conducted on chemical workers exposed to dioxins, revealing relatively mild acute effects. The observed responses include chloracne and, at high levels of exposure, a general sense of

fatigue or malaise, disturbances in the responses of the peripheral nervous system, and liver toxicity, including changes in many enzyme levels and, in some cases, enlargement of the liver. These conditions generally subsided after a few years.[33]

Although more than 800 workers have been exposed to dioxin in industrial accidents since 1949, no clear case of human death has been shown to be the result of dioxin exposure. However, recent studies have revealed that dioxin disturbs various aspects of sexuality, has subtle endocrine, developmental, neurological, and immunological effects, and is a potent carcinogen.[30] The above-mentioned studies on monkeys, showing a connection between endometriosis and dioxin,[31] led to research into the connection in the more than 5 million women in the U.S. with the disease. The results obtained from the studies have convinced many researchers that what is observed in animal studies also apply to humans.

Recently, researchers in both Milan, Italy, and at the Centers for Disease Control and Prevention in Atlanta, Georgia, reported that exposure to high levels of PCDDs in both parents was linked to an excess of female offspring. As mentioned earlier, an industrial accident in July 1976 released kilogram quantities of PCDDs near Seveso, Italy. Researchers found that, in the zone where the population was most heavily exposed to TCDD, 26 male babies and 48 female babies were born in the period from nine months after the accident until December 1984. Ordinarily, about 106 males are born for every 100 females. The ratio of males to females returned to normal between 1985 and 1994. The half-life of PCDDs in adults is about 8 years, so it can be assumed that about half of the PCDD was cleared from exposed adults by 1985. No males at all were born to parents who both had measured PCDD blood levels of 100 ppt or higher.

13.6.4 Gene Regulation by Dioxins

The similarity of biological effects of several classes of polychlorinated hydrocarbons, including PCDDs, led to the hypothesis that these compounds may act through a specific receptor.[29] Experiments with mice showed that dioxin induces the cytochrome P450 system and its associated enzymes. Researchers subsequently found that this response is governed by a single autosomal gene, with a gene locus that codes for the *Ah* receptor protein. The *Ah* receptor protein preferentially binds to arylhydrocarbons.[29] A similar receptor has been discovered in human cells.[34] The presence of the *Ah* receptor makes an organism more sensitive to several effects that dioxins and other PCDDs elicit, such as enzyme induction, carcinogenesis, and immunotoxicity. Different *Ah* receptor levels in different animals and genetic strains may explain why dioxin evokes biological responses at different dose levels.[29] These discoveries support receptor-medicated specificity of response.

Current understanding of a probable mechanism of gene regulation by dioxins may be summarized as follows:

1. TCDD first enters the cell through passive diffusion, then binds to the *Ah* receptor, forming a receptor complex TCDD–*Ah*.
2. The TCDD–*Ah* undergoes an unknown transformation or activation step, and can subsequently be translocated into the nucleus.
3. In the nucleus, the complex binds to specific regions of core DNA, called dioxin responsive elements (DREs).
4. Binding of the complex to DREs results in increased transcription of several genes.
5. The transcribed mRNA is then translated in the cytosol, resulting in the synthesis of cytochrome P450 enzymes.

This is considered the primary biological response (Figure 13.11). Secondary biological responses include perturbation of hormone systems and altered patterns of cell growth and differentiation.[29] Studies show a high correlation between laboratory animals and human responses. As mentioned previously, dioxin is now considered a carcinogen, although it does not damage DNA as most carcinogens do. By attaching to the *Ah* receptor and entering the nucleus, dioxin switches on genes that control cell growth and proliferation. Dioxin is also a cancer promoter as it can trigger DNA damaged by other carcinogens to start producing abnormal cells. Therefore, dioxin is considered a potent carcinogen because it can cause a wide variety of cancers, rather than a specific type.[30]

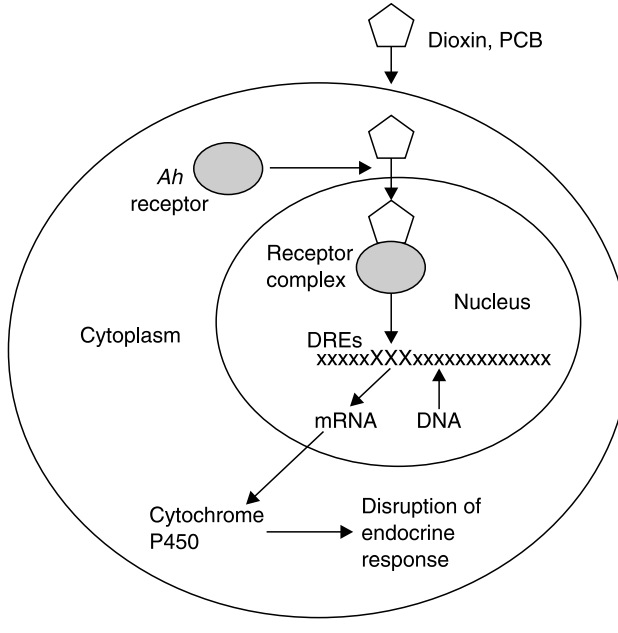


Figure 13.11 Proposed mechanism by which dioxins and PCBs effect endocrine disruption.

13.6.5 Environmental Degradation of TCDD

Although pure TCDD is extremely persistent, it is not stable as a contaminant in thin herbicide films exposed to outdoor light. Research shows that herbicide formulations containing known amounts of TCDD and exposed to natural sunlight on leaves, soil, or glass plates lose most or all of the TCDD within a single day.[36] It is agreed that three factors are required in order for dioxin to break down: dissolution in a light-transmitting film, the presence of an organic hydrogen-donor (such as a certain solvent or pesticide), and UV light.

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13.8 REVIEW QUESTIONS

1. What is the mode of action of most insecticides?
2. What are the characteristics of DDT?
3. Compare the characteristics of organophosphates and organochlorines.
4. Why is DDT persistent in the environment?
5. Which are more toxic to humans, organophosphates or organochlorines?
6. What is the mode of action of organophosphates in insects?
7. What are the reasons for organophosphates to be more widely used than organochlorines?
8. Describe the action of acetylcholinesterase (AChE).
9. Which of the following is (are) AChE inhibitor(s)? (a) DDT, (b) parathion, (c) carbamate.
10. What is meant by yu-sho or "oil disease"?
11. Female victims of PCB poisoning exhibit lowered serum estradiol levels. Explain why.
12. What properties do PCBs share with DDT?
13. What are the environmental sources of TCDD?
14. List the major symptoms exhibited by animals exposed to TCDD.
15. What types of cancer does TCDD elicit?
16. What is the current understanding of the mechanism by which dioxin is involved in gene regulation?

Chapter 14

Endocrine Disruption

14.1 INTRODUCTION

One of the most pressing environmental issues facing environmental toxicology is concerned with endocrine disruption. It is generally perceived that certain anthropogenic chemicals that can interact with and disrupt the endocrine system, and that exposure to them may cause some forms of endocrine malfunction. They therefore pose serious health problems for humans, wildlife, and fisheries.[1,2]

Chemicals that can induce endocrine disruption are called *endocrine disrupters* (EDs) or *endocrine disrupting chemicals* (EDCs). A broad definition of EDs is that they are exogenous chemical agents that interfere with the synthesis, secretion, transport, binding, action, metabolism, or elimination of natural hormones.[3] They are a group of chemicals with diverse structures. They include chemicals used widely in the past in industry and agriculture, such as organochlorine pesticides and polychlorinated biphenyls (PCBs), and chemicals that are currently in use, such as plasticizers and surfactants. Many of the known EDs are estrogenic, affecting reproductive functions in particular. Certain EDs are capable of mimicking the actions of progesterone, whereas others are antiestrogenic or capable of acting on the thyroid. Because of the persistent and lipophilic nature of most xenobiotic estrogens and their metabolites, many EDs bioaccumulate and biomagnify, potentially inducing adverse effects in living organisms.

14.2 REVIEW OF HORMONAL FUNCTION

Before discussing specific aspects of EDs, it is important to briefly review how hormones function in the body. Hormones are specific organic substances produced by the endocrine system. They are transported by body fluids, and produce specific effects on the activities of cells remote from their points of origin. The hormones that have received the most attention in recent years are estrogen and androgens. Most hormones are comprised of steroid, and are generally referred to as steroid hormones. These hormones are formed from cholesterol through a complex biochemical pathway; Figure 14.1 shows the main steps involved in this pathway.

Estrogen is one of the steroid hormones. It is a sex hormone, as are estradiol, estriol, and estrone. Estrogen is generally considered a female hormone, but it is produced in both males and females, although the quantities

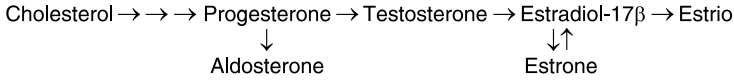

Figure 14.1 Main steps involved in steroid hormone synthesis.

produced in females are much greater than those in males.[4] Estrogen, produced particularly in the ovaries, is characterized by its ability to promote estrus and stimulate the development of secondary sex characteristics in the female. Estrogen also refers to a substance occurring naturally in plants or made synthetically (as DES) that has similar biological activity. Androgen, also a sex hormone (as androsterone and testosterone), is produced particularly in the testes and adrenal cortex, and is usually characterized by its ability to stimulate the development of sex characteristics in the male. Androgen also refers to a synthetic compound having similar biological activity. Figure 14.2 shows the structures of some steroid hormones.

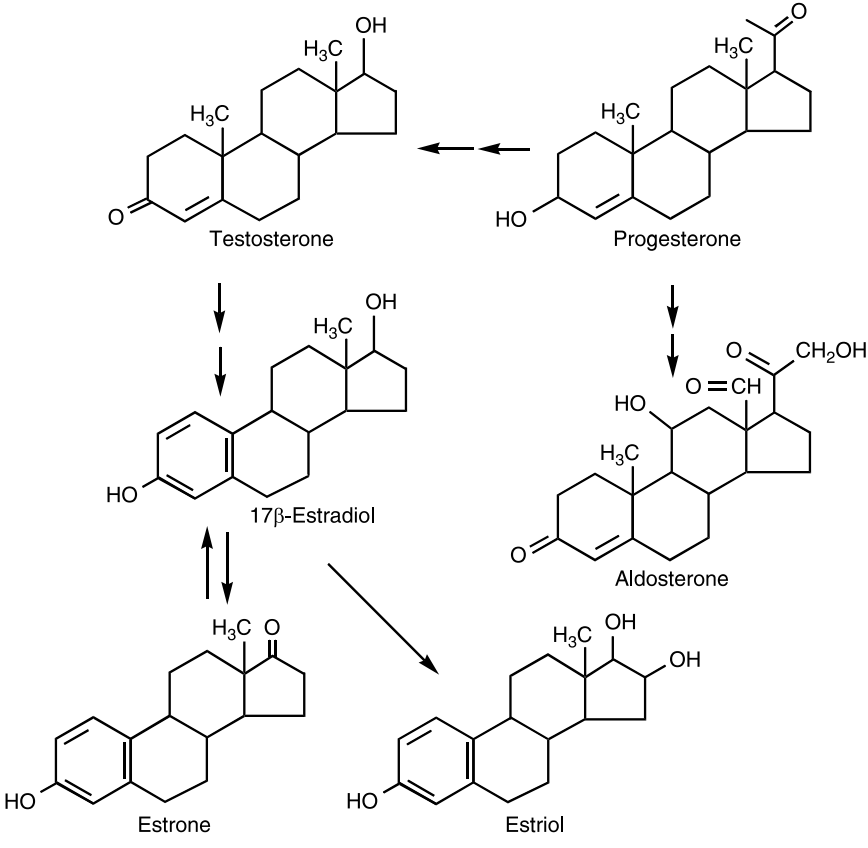

Figure 14.2 Structures of some steroid hormones.

The production of hormones is regulated by a complex negative-feedback pathway, which is turned on and off in response to changes in hormone levels. When hormone production peaks, the hormone acts as an inhibitor and causes the pathway to shut down.[4] Once hormones have been produced they travel through the bloodstream to target cells, where they attach to a receptor protein, forming a hormone–receptor complex that then enters the cell nucleus and binds to the DNA. Transport of the hormone and hormone–receptor complex to the DNA of a cell can occur in one of three ways, depending on the type of hormone. A hormone may bind to a receptor protein, which carries it to the cytoplasm of the cell. A hormone may move directly into the nucleus, where it binds to its receptor protein and initiates transcription of messenger RNA (mRNA). The lipophilicity of androgens and estrogens should facilitate their passage across the cell membrane to the cytoplasm. In the case of a hydrophilic hormone, such as a peptide, which cannot pass through the plasma membrane, the molecule binds to receptor proteins on the surface of the cell.[4] Binding to the protein receptor causes a change in the shape of the protein and induces a series of events in the cytoplasm. For example, protein receptors are often composed of several subuits – when such a receptor forms a complex with an incoming hormone, it may release some of its subunits (Figure 14.3).

Chemicals may be activated or deactivated as the signal is passed from one molecular receptor to another. Hormones initiate cellular and physiological changes by altering transcription of specific genes within the cell nucleus. In some cases, the hormone–receptor complex may inhibit transcription. It should be pointed out that only small amounts of hormones are necessary for inducing vital cellular and physiological responses. An organism will, therefore, be sensitive to any changes in the amount of hormone that is produced or that enters the cytoplasm. Furthermore, the interaction between the hormone and

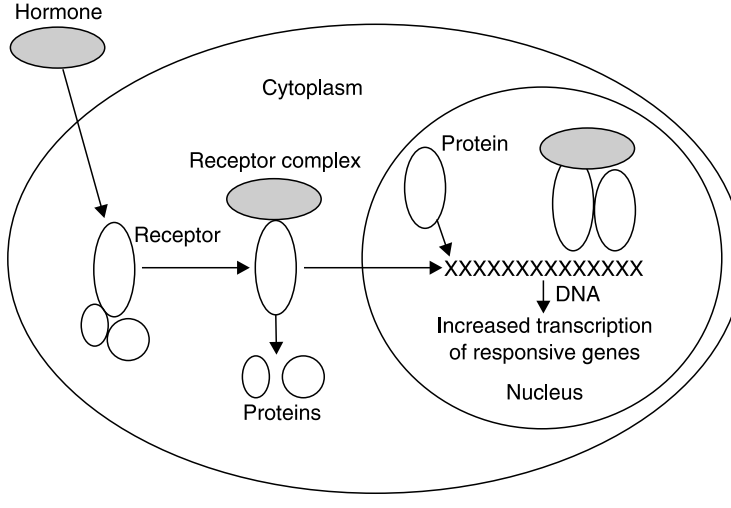

Figure 14.3 Regulatory role of steroid hormone.

the receptor is very precise, and constitutes the reception of a chemical message by a particular cell. The reaction to the interaction of the hormone and the receptor is specific to the type of cells involved.[5] Figure 14.3 shows the interaction between a hormone and its protein receptor in a cell.

14.3 CHARACTERISTICS OF ENDOCRINE DISRUPTERS

Many EDs have been identified. They include pesticides (including herbicides and fungicides), plasticizers, surfactants, organometals, halogenated polyaromatic hydrocarbons, and phytoestrogens. Representative examples are presented in Table 14.1. An important characteristic of environmental hormones is the lack of structural similarity with the estrogen itself. For example, Kepone (Figure 14.4) does not have structural resemblance to 17β-estrodiol (Figure 14.2), a representative estrogen, but manifests a potent endocrine disrupting effect. A second characteristic is that, unlike many other types of environmental pollutants, environmental hormones do not alter genes themselves, but may change the way they are expressed.[6]

Table 14.1 Examples of Endocrine-Disrupting and Estrogen-Like Pesticides

	Estrogen-disrupting pesticides	Estrogen-like pesticides
Herbicides	2,4-D	Atrazine
	2,4,5-T	
	Alachlor	
	Amitrole	
	Attrazine	
	Metribuzin	
	Nitrofen	
	Triflurafin	
Insecticides	i-Hexachlorocyclohexane	i-Hexachlorocyclohexane
	Carbayl	Kepone
	Chlordane	1-Hydroxychlordane
	DDT and its metabolites	*p,p′*-DDT
	Endosulfan	*o,p′*-DDT
	Heptachlor	*o,p′*-DDE
	Heptachlor-epoxide	
	Lindane	*p,p′*-DDE
	Methomyl	DDT
	Methoxychlor	DDE
	Mirex	Endosulfan
	Oxychlordane	Heptachlor
	Parathion	Methoxychlor
	Synthetic pyrethroid	Toxaphene
	Toxaphene	
Others	Nonylphenol (NP)	

Source: Adapted from *Encyclopedia Britanica Online.*

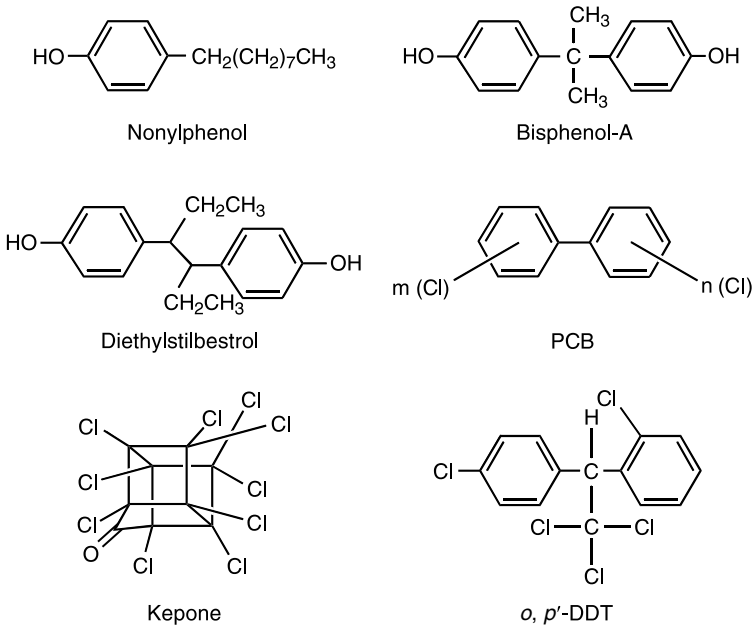

Figure 14.4 Structures of some endocrine disrupters.

Exposure to environmental EDs has been associated with a variety of abnormalities in wildlife and fish. Some examples include:

- abnormal thyroid function in birds and fish
- decreased fertility in birds, fish, and mammals
- decreased hatching success in fish and birds
- demasculinization of male fish, birds, and mammals
- defeminization and masculinization of female fish, gastropods, and birds
- alteration of immune function in birds and mammals

As mentioned previously, hormone–receptor interaction is quite specific. The manifested specificity is thought to result from two basic factors: the conformation of the receptor, and the three-dimensional structure of the xenobiotic and its resemblance to a natural ligand. For instance, studies were carried out to test the inhibitory effect of DDT and DDD isomers on the binding of tritiated estradiol ([^{3}H]-17β-estradiol) to alligator estrogen receptor. Results showed that both *o*,*p*′-DDT and *o*,*p*′-DDD manifested a potent inhibitory effect, but the close isomers *p*,*p*′-DDT and *p*,*p*′-DDD did not.[5]

Three characteristics are associated with the toxicity of EDs. First, EDs have high lipophilicity, and can therefore accumulate in lipid-rich cellular components, such as the membranes. Second, EDs are able to irreversibly bind to macromolecules such as DNA. Third, EDs are able to reversibly react at specific sites of the receptors and enzymes. The overall toxicity of EDs is the result of the combined effects of these three factors.[5]

14.4 MODE OF ACTION

Toxicants that are EDs are known to act in several basic ways. First, the toxicant may mimic the natural hormone and produce a structural change of the receptor, initiating a response (Figure 14.5a). Toxicity may then arise from an excess of gene products or from inhibition of transcription at inappropriate times. As a result, males, for example, may become feminized in the presence of an estrogen mimic. Second, the xenobiotic may bind to a hormone receptor, inducing changes in the transcription of mRNA, followed by altered gene expression. By binding to hormone receptors, EDs can block the action of a natural hormone, prohibiting it from binding to the receptor. In this case, the xenobiotic may bind to the active site of the receptor and prevent the natural hormone from attaching to the active site. In this way, the xenobiotic not only occupies the active site of the protein receptor, but also prevents the conformational changes necessary for correct hormonal response (Figure 14.5b). Xenobiotics that act in this way are called *estrogen antagonists*. An example of the outcome of such alteration is masculinization of the female.[5] As noted in Chapter 13, an ED such as 2,3,7,8-TCDD may interact with an aryl hydrocarbon (*Ah*) receptor, disrupting natural hormone function in the cell.

Other mechanisms of action include alteration in hormone synthesis, metabolism, and transport, as well as effects mediated through changes in the hypothalamic–pituitary–gonadal axis.[7] For example, a recent report indicates

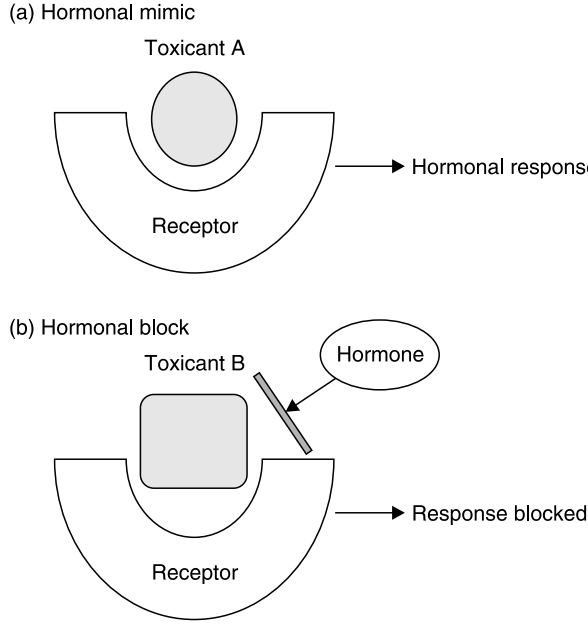

Figure 14.5 Proposed mechanisms of the actions of (a) a hormonal mimic, and (b) a hormonal block.

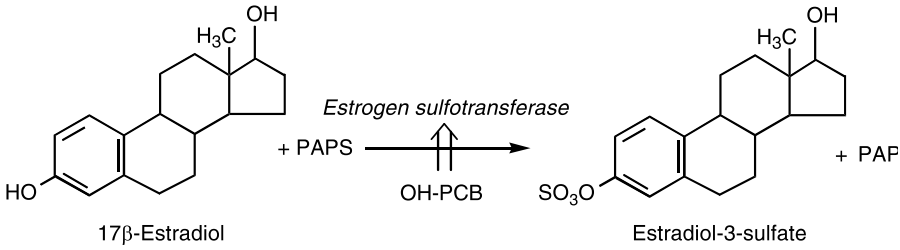

Figure 14.6 Hydroxylated PCB (OH-PCB) inhibits the catabolism of estradiol by estrogen sulfotransferase. (PAPS = 3′-phospho-adenosine-5′phosphosulfate).
Source: Adapted from Song, W.-C., *Ann. N.Y. Acad. Sci.*, 948, 43, 2001.

that some EDs may cause endocrine disruption by enhancing endogenous estrogen through inhibition of the enzymes involved in the biotransformation and inactivation of the estrogen.[8] According to the report, certain hydroxylated PCBs (OH-PCB) are potent inhibitors of estrogen sulfotransferase (EST) in humans. EST is a cytosolic enzyme which catalyzes the conversion of estrogen, such as 17β-estradiol into 3-sulphonyl estradiol (Figure 14.6). The conversion results in inactivation of the hormone. The sulfonyl group needed for the conversion is provided by 3′-phopho-adenosine-5′-phosphosulfate (PAPS). The enzyme (EST) has a high affinity for estrogens and is expressed in several estrogen target tissues, including the male and female reproductive systems.[8]

The observation that EST serves as a target for OH-PCB has a significant implication. The finding demonstrates that an environmental chemical does not have to interact with the estrogen receptor itself to cause endocrine disruption. In other words, a xenobiotic such as OH-PCB can inhibit the enzyme EST in estrogen target tissues and induce an increase in endogenous estrogen activity and toxicity.[8] This finding provides a new paradigm in explaining the endocrine disrupting potential of environmental chemicals that have low or no binding affinities for steroid hormone receptors.[8]

14.5 EXAMPLES OF ENDOCRINE DISRUPTION

The widespread presence of environmental estrogens and the possibility that their degradation products will persist in the environment, combined with the potential for inadvertent exposure of humans and wildlife to EDs, raise significant safety issues. Although most of the pesticides show weak endocrine-disrupting activity, some researchers consider it still possible that chronic exposure to those pesticides may lead to toxicity. A large number of findings have been reported in recent years concerning xenobiotics causing endocrine disruption. Several examples are reviewed briefly in the following sections.

14.5.1 Induction of Developmental Toxicity

Induction of developmental toxicity is viewed by many researchers as the most important action of EDs. The chemical classes that have been shown to induce developmental toxicity include pesticides, herbicides, fungicides, plasticizers, surfactants, and halogenated polyaromatic hydrocarbons. For example, a specific ratio of estrogen to androgens is necessary for sexual differentiation. Endocrine disrupters can perturb the ratio of estrogen to androgens in the developing fetus of humans and animals.[6] This perturbation may result in offspring with two sets of partially developed sexual organs, termed *intersex*, or with a single set that is improperly or incompletely developed.[2]

The Lake Apopka alligator episode has provided a unique example suggesting that environmental toxicants can potentially induce developmental toxicology in wildlife.[6,7,9] Lake Apopka, in central Florida, is situated in an area adjacent to a chemical plant. The area became contaminated with high levels of DDT and its metabolites after leakage from the chemical plant between 1970 and 1980. Subsequent comparative studies showed that male juvenile alligators from Lake Apopka had significantly smaller reproductive organs and lower concentrations of plasma testosterone compared with alligators from several less-polluted lakes. Although these findings could not be linked quantitatively to current pesticide levels in Lake Apopka, it was suggested that the observations could reflect past exposures of young alligators to chlorinated hydrocarbon pesticides.[7]

14.5.2 Estrogen Mimics

Estrogen mimics are a diverse group of chemicals that have no obvious structural similarity. Examples of estrogen mimics include DDT, DDE, Kepone, dieldrin, dicofol, PCBs, and methoxychlor. These chemicals have several common characteristics, such as persistency, high lipophilicity, and accumulation in adipose tissue of animals and humans over a long period of time. The majority of these chemicals appear to attach themselves to estrogen receptors and mimic the action of the body's natural estrogen, or block the action of natural estrogen (as mentioned previously). In addition, estrogen mimics may interfere with the normal metabolism of estrogen in the body.

Most of the estrogen mimics are able to cross the placental barrier, and so pass from the mother to the developing fetus. It is important to note that the amount of natural estrogen in the mother is usually much greater than the amounts of estrogen mimics. However, because most of the sex-hormone-binding protein in the blood is used in binding the natural estrogen, it is thus unable to bind estrogen mimics, leading to increases in the effective dose of the mimics and thus their toxicity.[5,9]

14.5.3 Induction of Sterility

A large number of reports have indicated that sperm counts in men have decreased about 50% worldwide since 1940, and that environmental estrogens are suspected of being involved. A significant decrease in sperm counts coincides with increased use of estrogenic chemicals during the past 50 years.[6,9] For example, a spill of Kepone, one of the cyclodienes (Figure 14.4), occurred in 1975. The spill resulted in a decreased sperm count in men exposed to the chemical. Subsequent studies showed that Kepone, which does not have a structural similarity with natural estrogen, is indeed a weak estrogen.

According to news media reports, scientists at King's College in London, U.K., have recently reported a direct link between the fertilizing ability of sperm and the presence of endocrine disrupters, including nonylphenol (Figure 14.4), in the environment. The researchers indicated that their study with mice was the first to provide both indirect and direct evidence that environmental estrogens significantly affect the fertilizing ability of sperm. According to the researchers, the estrogenic effect identified in the mouse sperm will be replicated in humans.

Diethylstilbestrol (DES)(Figure 14.5) exposure has been shown to induce malformations and adverse functional alterations of the brain and the male and female reproductive tracts. In animal experiments, exposure to higher levels of DES (10 to 100 μg/kg) resulted in total sterility of female offspring. This was due in part to structural abnormalities of the oviduct, uterus, cervix, and vagina, and to depletion and abnormalities of ovarian follicles.

Newborn rats treated with DES (10 μg/animal) postnatally on alternate days from the second day to the twelfth day were shown to delay the establishment of the blood–testis barrier for several weeks. In addition, the diameter of the seminiferous tubule in DES-treated rats was one half that of the control animals'. The mechanism involved in the observed delay is not clear.[10]

The impact of EDs on humans was first demonstrated by the observation of DES-induced cancer in young women. Eight cases of clear-cell adenocarcinoma (CCA) of the vaginas were identified in women who had been exposed to DES *in utero* one to two decades earlier. DES is a synthetic estrogen and was prescribed to pregnant women in the 1950s and 1960s to prevent miscarriage. According to a report by the National Research Council, more than 300 cases of CCA have been shown with the same health problem, caused by *in utero* exposure to DES. Reproductive tract abnormalities were also observed in males exposed to DES *in utero*. It is interesting to note that no abnormalities were observed in the pregnant women who had received DES.[7]

14.5.4 Antiandrogens

Chemicals that can bind to the androgen receptor (AR) without activating it, and simultaneously prevent binding of true androgens, are called *antiandrogens*. Principal manifestations of developmental exposure to an antiandrogen

are generally restricted to males, and include hypospadias (an abnormality of the penis in which the urethra opens on the under surface), retained nipples, reduced testes and accessory sex gland weights, and decreased sperm production. There are indications that birth defects in the male reproductive tract have increased over the past several decades.[9] Examples of antiandrogens are the fungicide vinclozolin and the DDT metabolite *p,p′*-DDE. *o,p′*-DDT has weak estrogenic activity.[3]

14.5.5 Induction of Imposex

Imposex is a condition in which females develop part of the male reproductive system, such as a penis and a vas deferens, which effectively prevents reproduction. For example, tributylitin (TBT) compounds have caused the disappearance or decline of the dog-whelk snail along the British coast. Marine snails in the Northeast Pacific have been found with signs of imposex caused by TBT pollution.[11,12] Fish near sewage treatment plants in the U.K. develop hermaphroditic characteristics, thought to be caused by the widespread use of contraceptive pills and the subsequent release of ethynylestradiol (via the sewage treatment plants). Earlier studies found that female mosquito fish downstream from pulp and paper mills in Florida were masculinized and developed male sex organs.[11]

The precise nature of imposex as a manifestation of endocrine disruption is still the subject of discussion but is believed to involve the suppression of the enzyme aromatase. Aromatase catalyzes the conversion of androgens to estrogens. The net effect of the suppression of aromatase in affected animals is a shift in the hormonal balance towards androgens.

14.5.6 Hypothyroidism

The thyroid gland produces thyroid hormone, which is responsible for basal metabolism. *Hypothyroidism* refers to deficient activity of the thyroid gland, or a resultant abnormal state marked by lowered metabolic rate and general loss of vigor. Hypothyroidism therefore causes growth retardation, cognitive deficits, delayed eye opening, hyperactivity, and auditory defects in rodents. PCBs may act at several sites to lower thyroid hormone levels during development, causing body weight and auditory deficits.[11]

14.6 HORMONAL CANCERS

14.6.1 Introduction

EDs are suspected of causing various types of human cancer, including breast cancer, prostate cancer, and testicular cancer. Over the past 50 years, the incidence of prostate cancer in some countries has doubled, while that of testicular cancer has tripled. It has similarly been shown that since 1940 the

incidence of female breast cancer has risen in Western Europe and the U.S. [9] A number of studies have shown the presence of residues of DDT and other organochlorine pesticides in human breast milk and adipose tissue. Exposure to these pesticides has been implicated in breast-cancer risk. An estimated 211,300 new cases of invasive breast cancer were expected to occur among women in the U.S. during 2003. Breast cancer incidence rates have continued to increase since 1980, although the rate of increase slowed in the 1990s compared with the 1980s.[13]

An estimated 60 to 70% of human breast cancers are associated with sex-hormone exposure. Approximately 60% of all breast-cancer patients have hormone-dependent breast cancer, which contains estrogen receptors and requires estrogen for tumor growth.[11] The possible roles of estrogens in the development of breast cancer are still unclear.

DES is a potent ED and has been shown to be a transplacental carcinogen, i.e., a chemical that when given to the mother causes cancer in her daughter. As mentioned earlier, DES was found to be associated with vaginal cancer in some of the adolescent daughters of women who had taken the synthetic estrogen to prevent miscarriage. In addition, it brought about cellular changes in the vagina or Fallopian tubes of female offspring, as well as structural changes in the uterus. This demonstrates that synthetic estrogens are capable of affecting the development of reproductive system and subsequent adult health.

Another observation is the increase in the prevalence of endometriosis, the growth outside the uterus of cells that normally line the uterus. Endometriosis was previously a rare condition, but reportedly now afflicts five million American women. This is a painful disease that afflicts women in their reproductive years, frequently leading to infertility. The U.S. Environmental Protection Agency has reported that PCBs may be involved in the induction of endometriosis.[6,9]

14.6.2 Hormonal Cancers in Farmers

Several epidemiological studies have shown that cancer risks among farmers are increased compared with those for the general population.[14,15] The agents most suspected as being responsible for this trend are pesticides. However, there is a lack of data showing the involvement of individual agricultural chemicals in the development of cancers among farmers. Most of the studies that show an association between pesticide exposure and cancer have been conducted among pesticide applicators or workers in pesticide-manufacturing plants. For example, a weak but statistically significant association between area sprayed with herbicides and prostate cancer deaths of Canadian farmers was found in a retrospective cohort epidemiology study linked to the Canadian National Mortality Database.[3] In a 30-year follow-up study of coke oven workers, an association between coke oven emissions and significant excess mortality from cancer of the prostate has been observed. Furthermore, in some epidemiological and animal studies, there is some evidence for a role of the heavy metal Cd in prostate cancer etiology.[3]

In addition to cancer of the prostate, other types of cancers that have been reported to be associated with farmers include cancers of the testicle, ovary, breast, thyroid, and endometrium. However, many types of exposure are involved in farming, making it difficult to find out which exposure is associated with which cancer. Obviously, further studies are needed for clarifying the issue. It is encouraging to note that the U.S. Department of Health and Human Services and Department of Agriculture have allocated funds for an extensive study on the relationship between farmers and cancers.

14.7 TESTING ESTROGENICITY

A number of methods have been developed and used for studying the presence and action of EDs. One of these methods is the use of vitellogenin (VTG). VTG is a typical female protein, and is the complex phospholipoglycoprotein precursor of egg yolk synthesized by the liver in response to estrogen stimulation. Very little VTG can be detected in males and juvenile fish. Induction of this typically female protein in males has been widely used as a sensitive biomarker of estrogenic effects.[2] VTG can be measured relatively easily using enzyme-linked immunosorbent assays (ELISA) or radioimmunoassay (RIA) techniques.

The method has been used widely in various countries in the study of endocrine disruption. For example, high induction of VTG was detected in adult male swordfish from the Mediterranean, suggesting that these species are exposed to high toxicological risk in the Mediterranean.[16] Similarly, a study termed the Endocrine Disruption in the Marine Environment (EDMAR) program has been conducted in the U.K. since 1996 (following a similar earlier project). The results from these studies show that plasma VTG concentrations in male flounder remained elevated in several U.K. estuaries throughout the period covered by the study.[17]

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14.9 REVIEW QUESTIONS

1. What is a hormone? What is its function?
2. What are the differences between androgens and estrogens? How are they derived?
3. What are the function of androgens and estrogens?
4. Define the term "endocrine disrupters."
5. What are the general characteristics of endocrine disrupters?
6. Explain the suggested basic mechanisms involved in the action of endocrine disrupters.

7. What is meant by hormonal mimic?
8. What is meant by imposex?
9. Explain the toxicity of DES.
10. What is vitellogenin?
11. How is vitellogenin being used in laboratory and field studies?

Chapter 15

Mutagenic Pollutants

15.1 INTRODUCTION

A mutation is a process by which the hereditary constitution of a cell is altered, ultimately resulting in a genetically altered population of cells or organism. Although mutations can occur in the RNA of viruses and the DNA of cytoplasmic organelles, the mutations of greatest interest occur within genes in the nucleus of the cell.

The human body is estimated to contain more than 10 trillion cells, and at some stage in its life cycle each cell contains a full complement of the genes needed by the entire organism. Genes, composed of DNA in the nucleus of cells, are clustered together in chromosomes. In the chromosomes of all but the most primitive organisms, DNA is combined with protein. DNA, the molecular basis of heredity in higher organisms, is made up of a double helix held together by hydrogen bonds between purine and pyrimidine bases, i.e., between adenine (A) and thymine (T), and between guanine (G) and cytosine (C). Figure 15.1 shows the structures of the five bases in DNA and RNA, and the pairing of bases in DNA is shown in Figure 15.2. The highly specific complementarity of these bases enables DNA to act as a template for its replication by DNA polymerases, as well as the synthesis of RNA transcripts by RNA polymerases. For the information contained in DNA to be biologically expressed, the sequence of the nucleotides in a gene is converted into the sequence of amino acids in a protein. It is the amino acid sequence that determines the enzymatic and structural properties of the protein thus formed.

DNA clearly plays a pivotal role in the expression and perpetuation of life. However, it is also a critical target for the action of many mutagenic environmental chemicals; lesions in DNA may occur through the action of physical or chemical agents found in the environment. Occurrence of mutation, however, depends on the nature of the initial lesion and the response of cells to the DNA damage. If the damage is intermediate, the mutations resulting from

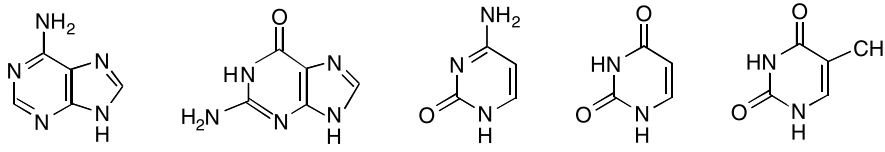

Figure 15.1 Structures of bases in nucleic acids.

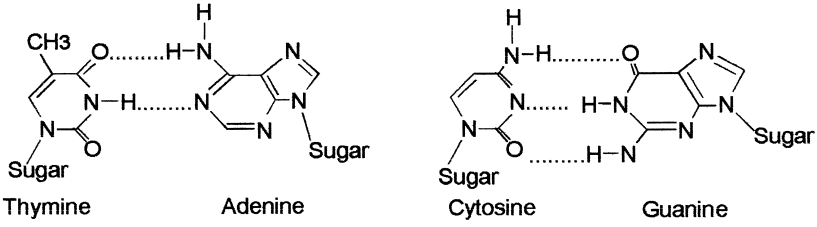

Figure 15.2 Pairing of bases in DNA.

it may be of immediate concern because mutations are implicated in pathogenesis of many inherited, somatic human diseases. However, if the damage is severe enough it can interfere with essential functioning of DNA and lead to the death of cells.

15.2 TYPES OF MUTATION

Mutations are often divided into two broad categories. The first category is *chromosomal aberration*, which refers to mutations that are cytologically visible. The second is *gene mutation*, mutations that occur at the submicroscopic level and are cytologically invisible.

15.2.1 Chromosomal Aberrations

A human cell normally has 23 pairs of autosomal chromosomes and a pair of sex chromosomes. In chromosomal aberration, mutation produces either a change in the number of chromosomes or a change in the structure of individual chromosomes. Changes that involve entire sets of chromosomes are called *euploidy*, whereas variations that involve only single chromosomes within a set are called *aneuploidy*. Alteration in chromosomal structure occurs when the chromosomes fracture and the broken ends rejoin in new combinations.

Major structural changes include *deletions*, *duplications*, *inversions*, and *translocations*. In deletion, a portion of a chromosome is lost (e.g., in ABCDE, the portion C is lost, becoming ABDE), whereas in duplication, an additional copy of a portion of the chromosome is inserted (e.g., ABCCDE). Deletions and duplications both upset the metabolic balance of an organism by altering the amount of gene product formed. An inversion is when the order of genes on a chromosome is reversed in one area (e.g., ABCDE becomes ACBDE). If a broken portion of a chromosome attaches itself to a second chromosome, it is termed a translocation (e.g., ABCDE $\rightarrow$ ABDE + $\underline{\text{C}}$, $\underline{\text{C}}$ + ABC $\rightarrow$ ABC$\underline{\text{C}}$). Because the position of a gene affects its regulation and activity, inversions and translocations may be detrimental.

15.2.2 Gene Mutations

In a gene mutation, an alteration occurs in the nucleotide sequence of a gene, which cannot be observed microscopically. Two subclasses of gene mutations have been identified: *point mutations* and *intragenic deletions*. Point mutations may involve the displacement of one nucleic acid base by another (base-pair substitution), resulting in substitution of one amino acid for another in the final gene product, thus altering cellular function. Alternatively, they may involve insertion or deletion of a nucleotide or nucleotides within a polynucleotide sequence of a gene (frameshift mutations). This leads to alteration in the nucleotide sequence, thus producing an incorrect gene product. An intragenic deletion occurs when a more-extensive deletion occurs within a gene, so that the informational material of that gene is essentially lost.

15.3 EFFECT OF MUTATIONS

Mutations often induce deleterious effects on the individuals or populations affected. While the effects of several individual mutagens (agents that cause mutations) are discussed later in this chapter, a general concept is addressed here. One of the concerns over mutagenic environmental agents is their relationship with cancer. As is widely recognized, the majority of human cancers appear to be related to environmental factors, and many mutagens have been shown to be carcinogens (cancer-causing agents). However, in the long term, the ability of different environmental agents to cause mutations (and teratogenic effects) may create a greater burden on society than cancer does because of the increased incidence of genetic disease and birth defects.

The total impact of genetic disease on national health is unknown. Autosomal dominant disorders have been shown to occur in 8 of 10,000 births.[1] A newspaper in British Columbia, Canada, reported that 9.4 individuals out of every 100 live births suffer from genetic diseases or disabilities, and that 2.7 of every 100 live births have disorders of unknown etiology that may be partly genetic.

Serious consequences can result if a mutation occurs in such a way that a hydrophilic amino acid is substituted for a hydrophobic residue in the resultant protein, or *vice versa*. Sickle-cell anemia, a hereditary disease, is a typical example. This disease is the result of a biochemical lesion caused by substitution of glutamic acid (a hydrophilic amino acid) for valine (a hydrophobic amino acid) in a chain of approximately 140 amino acids in human hemoglobin. This seemingly minor change produces abnormally shaped red blood cells that can no longer transport oxygen efficiently, leading to detrimental anemia.

Conversely, mutations may not necessarily produce deleterious effects on an organism. For instance, if a mutation occurs in such a way that only one amino acid along the backbone of a protein is incorrectly specified, the three-dimensional structure of the protein may not be greatly altered, allowing it to

function properly. This is usually the case when a hydrophilic amino acid residue in a protein is replaced by another hydrophilic amino acid, or a hydrophobic–hydrophobic replacement occurs.

Occasionally, a mutation may occur that results in the ability of a cell or a species to survive being improved. However, humans are highly developed organisms, and so when a mutation does occur, the probability is that it will be a deleterious one.

15.4 INDUCTION OF MUTATION

Commonly found mutagens that are of most concern to humans include: ultraviolet (UV) light, ionizing radiation, microtoxins, and organic and inorganic chemicals. Some common environmental mutagens and their sources are listed in Table 15.1.

15.4.1 UV Light

The region of the electromagnetic spectrum with wavelengths between 200 and 300 nm is of primary biological importance. The main reason for this is that DNA absorbs most strongly at 260 nm. It has been shown that mutations in microorganisms can be caused by irradiation of growth medium by UV light. Production of mutations by UV light, however, is strongly influenced by repair processes that reverse or remove induced photoproducts in DNA.

One of the most important ways in which the biological activity of DNA is altered by UV irradiation is thymine dimerization, a reaction in which two thymine molecules are fused together to form a dimer (Figure 15.3a). This dimerization may occur between adjacent thymine residues, or between two thymine residues across the chains (interchain dimerization). Dimerization results in disruption of hydrogen bonding between the bases in the DNA molecule (Figure 15.3b). Chain break (P - S - P - S) is another possible result. UV irradiation can also cause hydration of cytosine (Figure 15.4), which may also result in hydrogen-bond disruption. The effect of UV irradiation is not

Table 15.1 Common Environmental Mutagens

Mutagen	Sources
UV light	Sunlight
Ionizing radiation	Cosmic rays, medical x-rays
Nitrosamines	Pyrolysis products of tryptophan, broiled meat, beer and whisky
Benzo[a]pyrene	Cigarettes and wood smoke
Benzidine	Textile dyes, manufacture of paper and leather
Cr(VI), Hg	Metal alloys, mines
Hydrazine	Cigarettes and wood smoke
Malonaldehyde	Peroxidized polyunsaturated fatty acids
Vinyl chloride	Plastics
Aflatoxin B_1	Fungi-contaminated grains and peanut

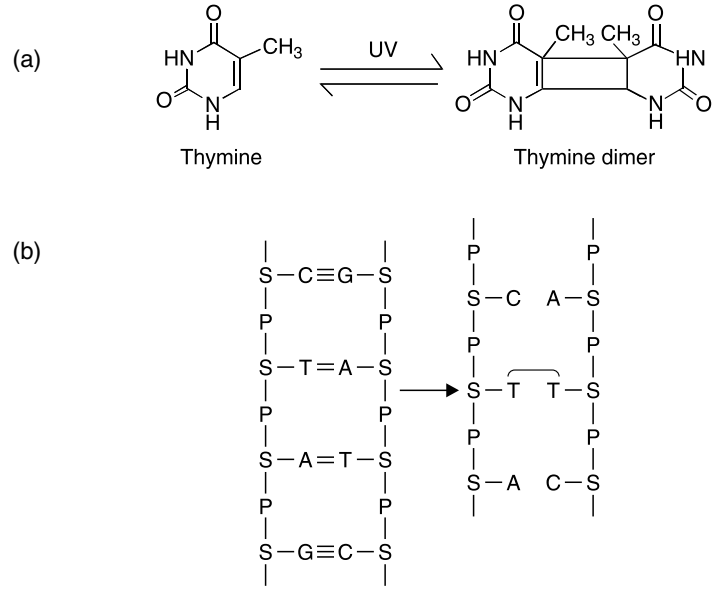

Figure 15.3 (a) UV radiation-initiated formation of a thymine dimer, and (b) interchain dimerization disrupts hydrogen bonding between DNA bases.

limited to DNA. Proteins and RNA outside the nucleus and other cellular components may also be affected.

15.4.2 Ionizing Radiations

Examples of ionizing radiations include x-rays, γ-rays, α-particles, high-energy neutrons, and electrons. Ionizing radiation produces various kinds of DNA damage, such as altering DNA bases, or producing single- or double-strand breaks in the phosphodiester chains of the DNA molecule, leading to frangmentation of the DNA. Such damages will consequently change the coding properties of DNA, resulting in induction of mutations.

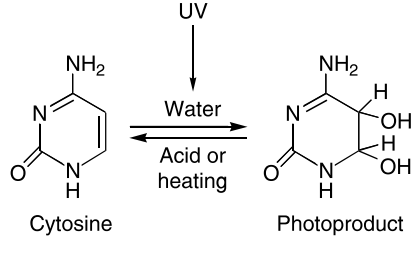

Figure 15.4 Hydration of cytosine.

15.4.3 Chemical Mutagens

Approximately 70,000 commercial chemicals are in use in the U.S., and this number is increasing by 1000 new compounds each year.[2] There are also many environmental chemicals that are of concern. Some of these are derived from the commercial chemicals, while others are produced from anthropogenic sources. Anthropogenic sources include: industrial processes involving combustion of fossil fuels, transportation,[3] open burning of scrap rubber tires, combustion of agricultural wastes (such as sugar cane, orchard prunings, and grain straws), municipal sewage sludges,[4] herbicide such as S-(2-chloroallyl) diethyldithiocarbamate (sulfallate),[5] and textile manufacturing.

Mutagenic compounds have been classified into seven major categories, based on their actions on DNA. The categories are: [6]

- alkylation
- arylation
- intercalation
- base analog incorporation
- metaphase poisons
- deamination
- enzyme inhibition

Table 15.2 summarizes the mechanisms involved in these categories. Some examples are given in the following sections.

15.4.3.1 Alkylating Agents

Alkylating agents represent the largest group of mutagens. They may carry 1, 2, or more alkyl groups in a reactive form, and thus are called mono-, bi-, or polyfunctional alkylating agents. These compounds can cause base alkylation, depurination, backbone breakage, or alkylation of phosphate groups. For example, most nitroso compounds are highly mutagenic (and carcinogenic) because of their ability to form electrophilic species. Figure 15.5 gives an

Table 15.2 Mechanisms of Action of Several Mutagenic Agents

Chemical action	Mechanism of action
Alkylation	Addition of an alkyl group ($CH_3CH_2CH_2$–, etc.) to a nucleotide
Arylation	Covalent bonding of an aryl group
Intercalation	The compound "wedges" into the DNA helix
Base analog incorporation	Base-pairing errors due to incorporation mispairing
Metaphase poisons	Interference with spindle formation and disruption of migration and segregation of chromosomes
Deamination	Removal of an amino group (NH_2) from adenine, cytosine, or guanine
Enzyme inhibition	Interference with biosynthesis of purines or pyrimidines and interference with repair

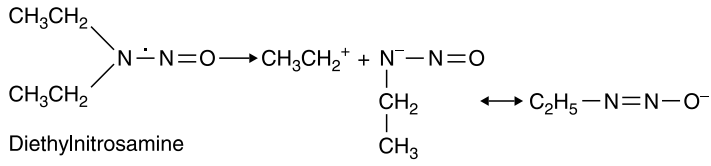

Figure 15.5 Diethylnitrosoamine, an alkylating agent.

example showing how diethylnitrosamine, a nitroso compound, can act as an alkylating agent. In this case, diethylnitrosamine is converted into two species, one of which is carbonium $CH_3CH_2^+$ ion. This ion may seek such nucleophilic sites as –N– or –S– on informational macromolecules, resulting in the covalent alkylation of a DNA base. For example, N-2 and N-3 of guanine (G) are highly susceptible to electrophilic attack. An alkylated G may not base-pair properly, or the information content of the molecule is altered in some way by the mutation. For instance, the alkylated G pairs with T instead of with C, thus causing transitional-type mutations. It is also possible for the alkyl group of N-7 to labilize the β-glycoside bond, resulting in depurination and leading to transition or transversion.

Some chemical mutagens, such as HNO_2, can react directly with nitrogenous bases of DNA. Other mutagens have structures that are similar to the structure of one of the bases; these are called *base analogs*. It is possible for these base analogs to be incorporated into a DNA molecule. For example, 5-bromouracil, in its normal (keto) form, hydrogen bonds with A (as would U or T), but in its enol form it base-pairs with G.

15.4.3.2 Intercalating Agents

Many planar aromatic hydrocarbons are thought to be able to position themselves (intercalate) between the flat layers of hydrogen-bonded base pairs in the interior of the DNA double helix, forcing it to partially uncoil. Such compounds are often called *intercalating agents*, and as a result of their action errors occur in the transmission of the genetic code. The chemical structures of several intercalating agents are given in Figure 15.6, and Figure 15.7 illustrates damage to DNA that can be induced by several of the agents discussed so far.

15.4.3.3 Metals

Many studies have shown the cytotoxic effects of a variety of metallic salts, which result in the denaturation of macromolecules. The reactions of metallic ions with nucleic acids are particularly important as some of the metals can contribute to mutagenesis and carcinogenesis. As noted in Chapter 12, exposure to mercury (Hg) results in decreased DNA content in cells. Hg also adversely affects chromosomes and mitosis, leading to mutagenesis.

The crucial factors in the toxic action of metals such as Hg may involve specific reactions with certain chemical groups in biomolecules, or with certain

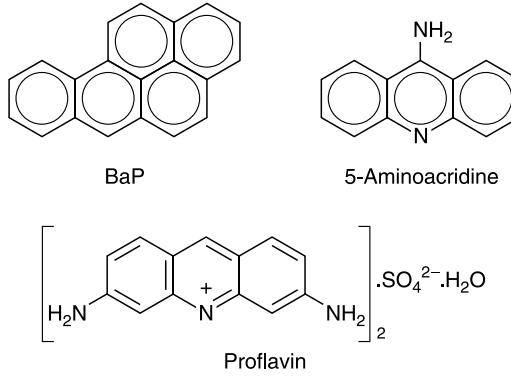

Figure 15.6 Examples of intercalating agents.

sites in tissues or organelles. Examples are given in Chapter 12, showing the interaction of Hg and Pb with the –SH group in proteins. A specific example showing the interaction of Pb with δ-aminolevulinic acid dehydratase (ALAD) in heme synthesis is also presented. As already noted, some toxic metals can compete with essential metals, such as magnesium (Mg), calcium (Ca), or zinc (Zn). These essential metals are required as cofactors in a number of enzyme systems; or they may contribute to stabilizing the structure of biomolecules.

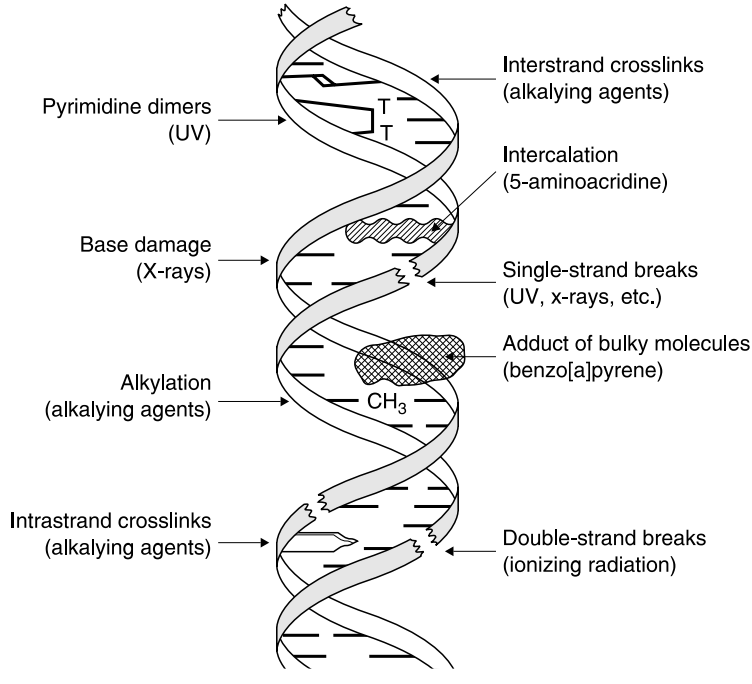

Figure 15.7 Mechanisms of DNA damage induced by various agents.

Research has shown that different metallic ions react with different ligands.[7] Mg^{2+} and Ca^{2+} ions, for example, bind to phosphate groups on nucleotides and tend to stabilize the DNA double helix, whereas Hg and silver (Ag) bind to bases, lowering the stability of the helix.

Several studies have shown that chromium (Cr)(VI) compounds induce chromosome aberrations and mutations in cultured mammalian cells.[8,9] Induction of DNA single-strand breaks and DNA–protein crosslinks by Cr(VI) compounds has also been reported.[10] Cr(VI) compounds can also inhibit the activity of such enzymes as glutathione reductase in cultured cells. After it enters the cell, Cr(VI) is reduced to Cr(III), through the intermediates Cr(V) and Cr(IV). This reduction process is accompanied by the formation of radical species such as active oxygen[11] as well as glutathionyl radicals.[12] These are considered to be responsible for the observed chromate-induced DNA damage. Interestingly, pretreatment with α-tocopherol (vitamin E) was found to reduce Cr-induced chromosomal aberrations. It is thought that because vitamin E is an efficient free-radical scavenger it may scavenge Cr(V) and free radicals.[10]

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15.6 REVIEW QUESTIONS

1. Define the term "mutation."
2. How are chromosomal aberrations different from gene mutations?
3. Match the following:
   A.
   (1) Inversion
   (2) Deletion
   (3) Translocation
   (4) Duplication
   B.
   (a) A portion of a chromosome is lost
   (b) The order of genes on a chromosome is reversed in one area
   (c) An additional copy of a portion of the chromosome is inserted
   (d) A broken portion of a chromosome attaches itself to a second chromosome
4. Which is more deleterious to an animal or a person?
   (a) Substitution of a hydrophobic amino acid with another hydrophobic acid
   (b) Substitution of a hydrophilic amino acid for a hydrophobic amino acid.
5. How does UV radiation affect DNA?
6. How do ionizing radiations affect DNA bases?
7. Briefly explain the phenomenon of dimerization. Which environmental agent(s) can cause it?
8. Describe alkylation as a mechanism of mutation induction.
9. Give an example to explain the term "intercalation."
10. How does Hg interact with the DNA helix?
11. Which is more toxic, Cr(III) or Cr(VI)? Why is Cr mutagenic?
12. Vitamin E appears to reduce the toxicity caused by Cr(VI). What is the possible mechanism involved in this phenomenon?

Chapter 16

Environmental Cancer

16.1 INTRODUCTION

Cancer refers to any of a group of diseases characterized by uncontrolled growth and spread of abnormal cells. In the scientific or medical community, the term *malignant neoplasm* (tumor) is often used in place of cancer. Malignant tumors develop most commonly in major organs, such as the lungs, liver, stomach, intestines, skin, breasts, or pancreas, but they may also develop in lips, tongue, testes, or ovaries. Cancer may also develop in the blood-cell-forming tissues of the bone marrow (the *leukemias*) and in the lymphatic system or bones.

In recent decades there has been growing concern about the possible effects of a large number of environmental toxicants on carcinogenesis. As noted in previous chapters, cancer incidence and mortality have increased dramatically over the past century. Researchers consider that there are two main reasons for the observed increase: the aging of the population, and an increase of carcinogens present in and released into the environment through human activities. Studies show that nearly 30% of the total mortality in many industrialized countries is attributed to cancer. In the U.S., cancer remains the number-two killer, accounting for nearly one fourth of all deaths. Despite the recent decline in the mortality rate, the total number of cancer deaths continues to rise as the elderly population increases. For example, the death toll in the U.S. in 1980 was 416,509, in 1995 it was 538,455,[1] and it is estimated to be 556,500 in 2003.[2]

One of the most common characteristics of the development of a neoplasm in an organism is the long period of time between the initial application of a carcinogenic (cancer-causing) agent, or carcinogen, and the appearance of a neoplasm. The latency period varies with the type of carcinogen, its dosage, and certain characteristics of the target cells within the host. In humans, cancer may not be manifested until at least 10 or more years after an initial exposure to a carcinogen.

16.2 CAUSES OF CANCER

Many factors can lead to cancer. These factors include: diet, smoking, alcohol, reproductive and sexual behavior, occupational hazard, geographical factors, and environmental agents. An estimate of the contribution of various agents or life styles to the cause of cancers is presented in Table 16.1. It is notable that

Table 16.1 Speculative Proportion of Cancer Deaths Attributed to Various Factors

Factor or class of factors	Percent of all cancer deaths
Diet	35
Tobacco	30
Reproductive and sexual behavior	7
Occupational hazards	4
Geophysical factors	3
Alcohol	3
Pollution	2
Industrial products	1
Medicine and medical procedures	1
Infection	10?
Unknown	?

Source: Adapted from USDHHS, *The Surgeon General's Report on Nutrition and Health*, U.S. Government Printing Office, Washington, D.C., 1988.

diet and smoking account for approximately two thirds of all cancers. Smoking is particularly implicated in lung and bladder cancers.

Although there are many theories concerning the causes of cancer, the fundamental principle underlying these theories is the alteration of the genetic material of the cell, the DNA. The various theories attempt to explain how this change is brought about. The DNA of a cancer cell is slightly different from that of a normal cell. This means that the sequence of the bases – adenine (A), guanine (G), thymine (T), and cytosine (C) – in a given strand of DNA is not the same as that of the bases in a normal cell. As mentioned in Chapter 15, these sequences dictate the sequences of the transcribed messenger RNA (mRNA), which in turn specify the kinds of proteins to be synthesized in a cell. Alteration in the DNA base sequence in cancer cells results in abnormal proteins. These new proteins influence the mechanisms of growth control in such a way that cell division continues indefinitely.

As discussed in Chapter 15, several types of DNA damage can occur. The most common ones include: single- and double-strand breaks in the DNA backbone, formation of crosslinks between DNA bases and between DNA bases and proteins, and chemical addition to the DNA bases. These alterations can result from exposure to radiation and to chemical, biological, and genetic factors (Table 16.2). For example, ionizing radiations, such as x-rays and γ-rays, can produce DNA single- and double-strand breaks and various forms of damage to bases. Ultraviolet (UV) light, which is a non-ionizing radiation, is capable of producing dimers. A variety of chemicals can cause DNA damage through base alterations. Alteration may be induced directly through formation of adducts, or indirectly through intercalation formed by a chemical between two bases. Many electrophilic chemicals can react with DNA, forming covalent additional products termed *adducts*. For example, alkylating agents can yield a reactive alkyl group that can react with base material, such as guanine, to produce an adduct.

Table 16.2 General Classification of Carcinogenic Agents

Class	Example
Radiation	Ultraviolet and ionizing radiations
Chemical	Polycyclic aromatic hydrocarbons, aromatic amines and halides, benzene, vinyl chloride, aflatoxin B_1, urethane, asbestos, certain metals, diet, and tobacco smoke
Genetic	Viruses
Biological	Transgenesis by enhancer–promoter–oncogene constructs

16.3 STAGES IN THE DEVELOPMENT OF CANCER

It is generally accepted that the pathway leading to *carcinogenesis* includes three stages: initiation, promotion, and progression (Figure 16.1).[3] *Initiation* results from a simple mutation in one or more cellular genes that control key regulatory pathways of the cell. It requires cell division for the fixation of the process. Unlike promotion or progression, initiation is irreversible in a viable cell.[4] The efficiency of initiation is sensitive to xenobiotic and other chemical factors, and the stage can be altered by both endogenous and exogenous factors. For example, a variety of chemicals in different tissues can inhibit the metabolism of a procarcinogen to an ultimate carcinogen (see Section 16.6), thereby blocking the initiation process. Initiators may also produce trans-

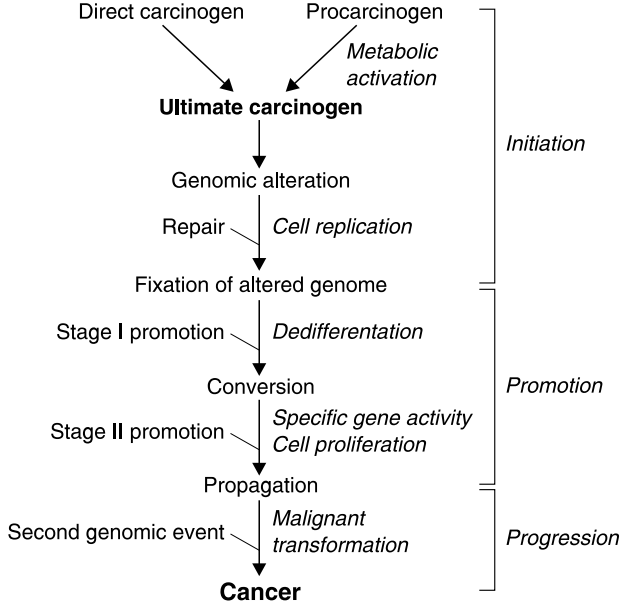

Figure 16.1 Three stages of carcinogenesis.
Source: Adapted from USDHHS, *The Surgeon General's Report on Nutrition and Health,* 1988.

formed cells that can persist for the life-span of an individual without producing cancer. In such cases, the damaged gene in the transformed cells remains recessive because the damaged gene does not express an abnormal protein.

Promotion results from the selective functional enhancement of signal transduction pathways induced in the initiated cell and its progeny by the continuous exposure to the promoting agent.[4] This stage involves gene activation, leading to the synthesis of the abnormal protein. Rapid cell division then occurs, which is accompanied by interruption of the organism's normal functions or health. Promotion then leads to the expression of the genetic changes as malignancy, which involves loss of control over cellular proliferation. Examples of promoting agents include: saccharin, butylated hydroxytoluene, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, see Chapter 13), and androgens and estrogens. In contrast to initiation, promotion is reversible. Therefore, if the promoting agent is withdrawn well before tumors are manifested, the appearance of tumors can be delayed or prevented. Furthermore, promotion may be continually modulated by various environmental factors, including frequency with which the promoting agent is administered, age and sex of the subject, hormonal balance, and composition and amount of diet. Research shows that many promoting agents exert their effects on the cell through mediation of receptor mechanisms.[5]

Some chemicals act as both initiators and promoters. Benzo(a)pyrene is such a chemical. In small doses it initiates genetic damage, and in higher or repeated doses, it enhances promotion.

Promoting agents involved in the onset of promotion do not cause cancer by themselves; they only have a specific impact on an initiated cell. Promotion is gradual, and some of the earlier steps are reversible. In the promotion stage, abnormal proliferation of the affected cell occurs, presumably because of a high concentration of growth factors or modified cell-surface receptors. If the damage to the gene is not drastic, most of the normal components of the cell will be produced and will be responsive to normal growth-inhibiting factors. Experiments with animals suggest that the time lapse between initiation and promotion is not critical. During the latter stage of promotion, however, cumulative genetic changes occur, leading to totally irreversible neoplastic transformation.

Progression results from continuing evolution of an unstable karyotype. This stage usually develops from cells in the stage of promotion, but, in certain conditions, it may develop directly from normal cells. The critical molecular characteristic of this stage is karyotypic instability, and morphologically discernible changes in cellular or genomic structure occur.[4] Furthermore, benign or malignant tumors may be observed in this stage. The growth of altered cells is sensitive to environmental factors during the early phase of progression.

16.4 METASTASIS

The most fearsome aspect of cancer is the spread of malignant cells from the primary site to other parts of the body – a process called *metastasis*. This is the late stage of the disease and is characterized by invasive activity and the appearance of a variety of cancer-cell types. Some of the cells that have the inherent ability to detach from the primary site eventually travel via the blood or lymph to start a secondary tumor at another site. Metastasis is the primary cause of the failure of treatment in cancer patients. The extent of the dissemination of the malignant cells is determined by the physiological condition of the host. During metastasis, continuous changes occur in the tumor, and the function and behavior of the tumor cells in the late stage are quite different from those in the early stage. Most frequently, the location of metastasis is in the organ or organs that are served by blood vessels from the original cancer site. Notably, growth and survival of a tumor require nourishment, which is provided by new blood vessels near the tumor site.

16.5 CLASSIFICATION OF CARCINOGENS

Carcinogens are divided into two groups: Part A and Part B (based on a list prepared by the U.S. National Toxicology Program, see Appendix 3). Part A refers to those agents that are ''Known to be a human carcinogen,'' whereas Part B refers to those that are ''Reasonably anticipated to be a human carcinogen.'' Examples of carcinogens belonging to Part A include: aflatoxins, inorganic arsenic compounds, asbestos, benzene, beryllium, coal tars, dioxin, diethylstilbestrol, tobacco smoking, steroidal estrogens, nickel compounds, radon, vinyl chloride, and UV radiation (see Appendix 3, Part A). More than one hundred agents are included in Part B (see Appendix 3, Part B).

As noted earlier, the basic changes in DNA that can lead to cancer, i.e., mutation, can be caused by many agents. These agents are generally divided into four categories: radiation, chemical, biological, and genetic (Table 16.2).[3] Although mutation does not necessarily result in cancer, cancer occurs if the proteins that are produced following mutation affect cellular growth-control mechanisms. The following section discusses in some detail the agents that can cause DNA damage. Emphasis is placed on radiation and chemical agents.

16.5.1 RADIATION

The process involved in radiation-induced DNA damage is complex and has received much attention over many years. As noted previously, ionizing radiation produces a wide variety of DNA lesions, including various base modifications, strand breaks, and DNA-protein crosslinks.[6] It was mentioned in Chapter 15 that absorption of short-wave UV radiation by DNA causes breakage in its strands, the opening of the rings of its bases, and the formation of thymine dimers.

UV radiation is the main cause of skin cancer. Increased UV radiation exposure– much of it is caused by sunbathing or tanning under a UV lamp – is the main contributing factor to the rising incidence of skin cancer worldwide. UV radiation induces formation of free radicals, especially reactive oxygen radicals. Of the three types of UV radiation (UV-A, -B, and -C), UV-B is the most harmful type. UV-B (which has a wavelength of 280 to 320 nm) is attenuated by the earth's ozone layer. Several other factors modulate the amount of UV radiation to which people are exposed, including time of day, season, humidity, and distance from the equator. Skin cancer risk is also affected by skin type; fair skin that freckles or bumps easily is at more risk than very darkly pigmented skin. People who live in sunny climates and have red or blond hair and blue or light-colored eyes are at especially high risk.

Among the photochemical reactions that take place when UV-B penetrates the skin is mutation of the DNA in skin cells. Humans have repair enzymes that can correct this damage, but mutations accumulate as the individual ages. An individual's lifestyle may also cause the repair system to eventually become overtaxed, resulting in skin cancer. Most researchers stress that the damage begins accumulating early – in childhood; by young adulthood about 50% of lifetime sunlight exposure may have already accumulated.

16.5.2 Chemical Carcinogens

The association between exposure to chemicals and cancer incidence was first reported in 1775 by the English physician Percivall Pott, following the observation of scrotal cancer in chimney sweeps.[7] With an increase in European industrial development during the 19th century, high rates of skin cancer were observed among workers in the shale oil and coal tar industries. In 1915, a group of Japanese scientists conducted experiments in which they painted rabbits with coal tar and induced tumors. This led to the knowledge that the compounds contained in the coal tar could produce cancer in animals. Several groups of organic compounds have now been recognized as carcinogenic to laboratory animals. These include polycyclic aromatic hydrocarbons (PAHs), aromatic amines, aminoazo dyes, nitroso compounds, benzene, and vinyl chloride.[8]

Many chemical agents that may be found in foods are also known to cause cancer. For example, aflatoxin B_1, which causes liver cancer in several species of test animals, is produced by *Aspergillus flavus* found in contaminated peanut or cottonseed meal. There are also naturally produced substances that are carcinogenic.

A number of inorganic substances have also been shown to induce cancer. These include some salts of arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr)(VI), nickel (Ni), and lead (Pb). It should be pointed out that some of these metals are essential nutrients for humans and animals. Trivalent Cr (Cr^{3+}) is one of these metals. As part of the glucose tolerance factor, Cr plays an important role in maintaining normal glucose metabolism in mammals.

Several chlorinated hydrocarbons and other chemicals have been identified as carcinogenic. These include 2,4-D, DDE, hexachlorocyclohexane, polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-*p*-dioxins (PCDDs) (see Chapter 13).

16.6 METABOLISM OF CHEMICAL CARCINOGENS

As shown in Figure 16.1, chemical carcinogens are divided into two broad classes: direct carcinogens and procarcinogens. *Direct carcinogens* are usually electrophiles, such as H^+, C^+, N^+, and can react readily with nucleophiles, such as proteins and nucleic acids. The main sites in these molecules where such reactions can occur are S, =N–, –C–OH, or –P–OH. Examples of cellular nucleophiles include some amino acids, such as methionine, cysteine, histidine, tryptophan, and tyrosine, and nucleic acid bases, such as adenine (N-1, N-3) and guanine (C-8, N-7, O-6). *Procarcinogens* are those agents that require biologic activation before becoming *ultimate carcinogens*. Compared with direct carcinogens, procarcinogens are relatively stable, and so many people may be environmentally or occupationally exposed to them. It is possible for people to ingest or absorb some procarcinogens, after which enzymes in the liver, lungs, or other organs convert them to their activated metabolites.

It is thought that most, and probably all, chemical carcinogens are converted by metabolism into electrophilic reactants that exert their biological effects by covalent interaction with DNA. Some examples of these reactants are shown in Figure 16.2. Several of these chemicals are discussed in some detail in the following sections. The discussion will focus on free radicals, DDT, vinyl chloride, nitrosamine, benzo[a]pyrene, and halogenated aromatic hydrocarbons.

16.6.1 Free Radicals

Reactive oxygen species, such as hydroxyl radicals ($OH^{\cdot}$), are produced during the enzymatic and chemical reactions of molecular oxygen in cells. Hydroxyl radicals are also produced when cells are exposed to ionizing radiation, tumor promoters, and chemical carcinogens. As mentioned earlier, reactive oxygen species can cause various lesions in DNA, by inducing damage to nucleic acids and altering their structures and function. Oxygen-induced lesions of nucleic acids include strand breaks[9] and base modification products. Alternatively, the $OH^{\cdot}$ free radical, formed through the reaction between superoxide free radical ($O_2^{\cdot -}$) and H_2O_2 (Reaction 16.1), is unique and can induce breaks in the phosphodiester bonds. Both single- and double-strand breaks can occur. In addition, the free radical can abstract H-atoms from the DNA helix.[10]

$$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + HO^- + HO^{\cdot} \qquad (16.1)$$

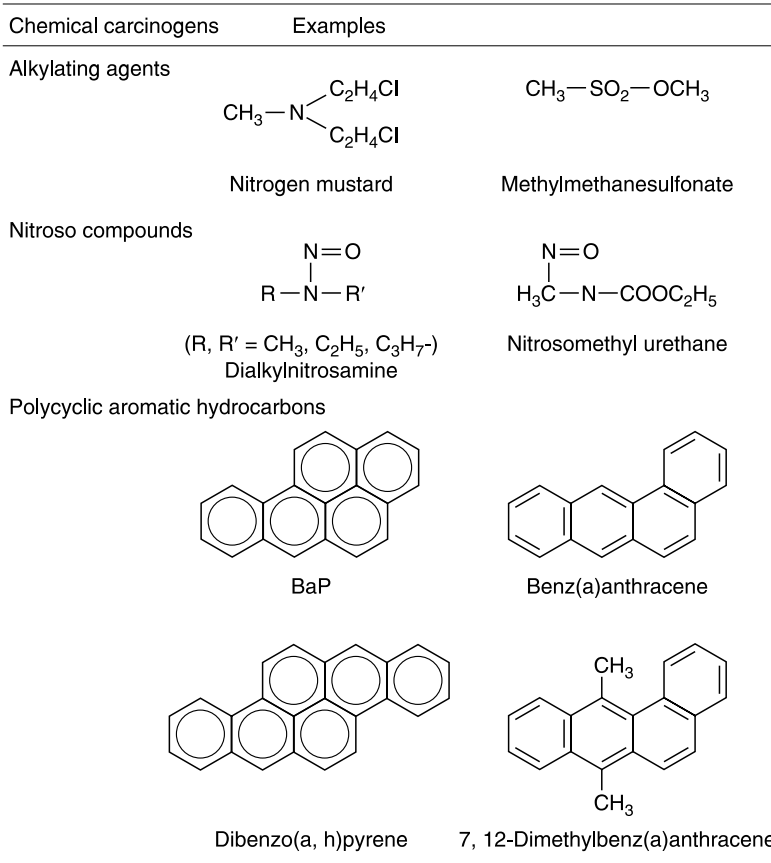

Figure 16.2 Some examples of chemical carcinogens.

16.6.2 DDT

DDT is one of the several pesticides that have been added to the long list of cancer-causing agents present in the environment. According to a report by the National Cancer Institute, women with high exposures to DDT may have a greater risk of developing breast cancer. Researchers at Mt. Sinai Hospital in New York City have found that women with blood levels of DDE (see Chapter 13) of 19 ng/ml have four times the risk of breast cancer compared with women with levels of 2 ng/ml.

It is suggested that DDE may cause breast cancer in two ways: it may induce cytochrome P450 enzymes, thereby altering the metabolism of toxicants, or it may act as an estrogen mimic and as such may disrupt the endocrine system through interaction with estrogen receptors (see Chapter 14).

16.6.3 Vinyl Chloride

Vinyl chloride, the common name for monochloroethene (CH_2=CHCl), is one of the most widely manufactured organic chemicals in the U.S. Vinyl chloride is a gas at ambient temperature, with a boiling point of 14°C, and exhibits a low solubility in water. While the vinyl chloride monomer itself is rarely used, it is polymerized with itself and other organic compounds to form many products, making it a very important chemical to industry and to consumers.

Among the many polymers that are derived from vinyl chloride, polyvinyl chloride (PVC) is the most common. PVC, as a solid material, is extremely adaptable and cost effective, and is used in numerous construction materials, home furnishings, packaging materials, automobile products, etc. Some examples of the products made of PVC are water pipes, raincoats, credit cards, wire coatings, and food packaging.

PVC production involves three stages: synthesis of vinyl chloride monomer from petrochemicals and chlorine, polymerization of vinyl chloride into PVC resin, and PVC fabrication. Environmental contamination occurs from these processes, although the extent of it varies with each stage. The contamination includes emission of vinyl chloride into the atmosphere, and surface and groundwater contamination resulting from sludge and wastewater discharge.

Vinyl chloride has been shown to be both mutagenic and carcinogenic. It is classified as a Part 1 carcinogen because sufficient evidence exists that the compound is carcinogenic to humans. This is highly important because only about 40 chemicals or chemical mixtures are classified as such.[11] Vinyl chloride causes liver cancer in both humans and laboratory animals. However, laboratory experiments with mice showed induction of not only liver cancer but also cancers of bone, skin, lung, brain, nephron, and mammary tissues.[11,12] In humans, vinyl chloride exposure may occur both occupationally and non-occupationally.

Vinyl chloride is metabolized by the hepatic cytochrome P450 enzymes to the carcinogenic epoxide form. Studies show that this metabolite is an ultimate carcinogen. It reacts with DNA, causing it to change its function. In the liver, the active epoxide may be further converted to chloroethane aldehyde. A molecule of glutathione can conjugate the aldehyde and the resultant conjugate may then be excreted (Figure 16.3).

16.6.4 Alkylating Agents

As noted in Chapter 15, alkylating agents are those chemicals that can react with DNA to produce alkylated DNA adducts. Several groups of organic compounds can be metabolized to alkylating agents. An example is N-nitroso compounds, which consist of nitrosamines and nitrosamides. Nitroso compounds are found in various types of food, particularly meat and meat products (e.g., fried and cured meat products) and cheese. Small amounts of the compounds have been shown to occur in beer, and tobacco smoke contains

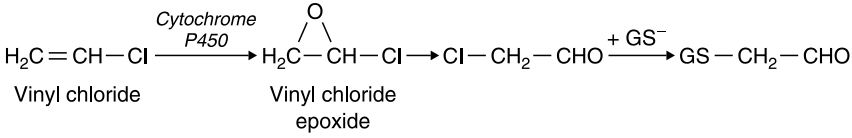

Figure 16.3 Metabolism of vinyl chloride by the cytochrome P450 system.

varying amounts. Industrial exposure to N-nitrosamines accounts for another environmental source. Occupation or industrial activities that may lead to exposure include metal cutting and rolling, leather tanning, rubber manufacture, handling of hydraulic fluids, and producing or using amines in the chemicals industry. In these activities, exposure is mostly via air and skin.[13]

The importance of nitrosamines as environmental carcinogens was first postulated in 1962. Subsequent studies demonstrated the endogenous formation of such compounds from precursor amines and nitrite *in vivo*. The endogenous formation of N-nitroso compounds from precursor amines and nitrosating agents, particularly nitrite, is unique among the various chemical carcinogens. Nitrosatable amine precursors, such as secondary and tertiary amines, are natural constituents of food or contaminants of food, such as some pesticides that can be nitrosated. Nitrite is the most important nitrosating agent and is present in some food products. However, nitrite can also be formed from nitrate in saliva and possibly in the intestines. The pathway leading to the formation of an alkylating agent from dimethylamine is presented in Figure 16.4. The first step is nitrosation in which dimethylamine reacts with nitrite to form dimethylnitrosamine, a nitroso compound. Metabolism of dimethylnitrosamine leads to the formation of a CH_3^+ radical, which can react with DNA, resulting in methylated DNA.

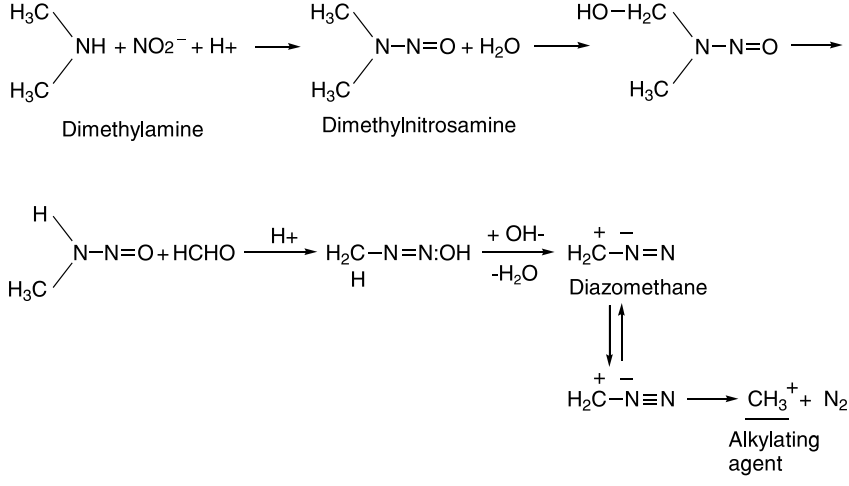

Figure 16.4 Activation mechanism of dimethylamine.

16.6.5 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds composed of two or more fused aromatic rings. They are emitted into the environment through both natural and anthropogenic combustion processes. The two main sources of natural PAH productions are volcanic eruptions and forest fires. Anthropogenic sources include combustion of fossil fuels by automobiles and other transportation systems, petroleum-refining processes, coking plants, asphalt production, industrial facilities that use fossil fuels, effluent disposal, oil spills and refuse burning. PAHs are, therefore, widely distributed in all parts of the environment – air, soil, water, and sediments. They are of major concern because they represent a potential human health hazard through contamination of food and drinking water supplies. Estimated carcinogenic PAH concentrations in various environmental media are presented in Table 16.3.[14]

16.6.5.1 Benzo[a]pyrene

Among the many PAHs, benzo[a]pyrene (BaP) (Figure 16.5) is probably the most widely known. In 1775, the British surgeon Percivall Pott first reported the relationship between the incidence of cancer of the scrotum among chimney sweeps and exposure to soot. Since then, many researchers have repeatedly shown the potent carcinogenic effect of BaP. Sources of BaP emission include burning of coal and refuse, residential furnaces, coke production, vehicle

Table 16.3 Estimated PAH Contents of Various Environmental Media

Environmental medium	PAH content
Outdoor air	2.6 to13.0 ng/m^3
Indoor air	1.5 to 13.0 ng/m^3
Surface water	8.0 ng/l
Groundwater	1.2 ng/l
Drinking water	2.8 ng/l
Rural soil	0.07 mg/kg dry wt
Urban soil	1.10 mg/kg
Road dust	137 mg/kg
Charcoal broiled or smoked beef	35 μg/kg
Pork	26 μg/kg
Poultry	12 μg/kg
Fish/shellfish	0.10 μg/kg
Smoked fish/shellfish	36 μg/kg
Green leafy vegetables	46 μg/kg
Grains	9 μg/kg
Fruits	2.4 μg/kg
Fluid milk	0.09 μg/kg
Fats and oils	66 μg/kg
Cheese	1.70 μg/kg
Alcohol beverage	0.08 μg/kg

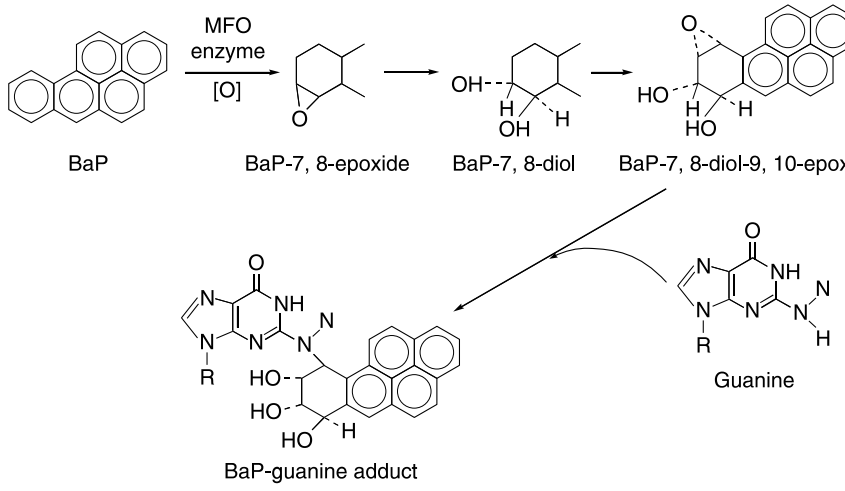

Figure 16.5 Benzo[a]pyrene (BaP) and formation of BaP–guanine adduct.

disposal (open burning), wood burning, and forest and agricultural refuse burning. BaP is found in most commercial motor oil, asphalt roofing and other construction materials, and tobacco smoke (Table 16.4).

Like most PAHs, BaP is ubiquitous in the environment, being found in air, water, soil, and food. According to Menzie et al. [14] the BaP concentrations in 58 prepared meals averaged 0.15 μg/kg, with a range of 0.005 to 1.17 μg/kg.

BaP can cause several forms of cancer, particularly cancers in the lung, intestine, kidney, and liver. It has been reported that the ultimate carcinogenic form of BaP is benzo(a)-7,8-diol-9,10-epoxide.[15] This active form of BaP is formed through cytochrome P450-dependent activation followed by several enzymatic steps. The resultant BaP metabolite then forms an adduct with the base guanine in DNA, altering its function. Figure 16.5 summarizes the activation process.

16.6.6 Halogenated Aromatic Hydrocarbons

As discussed in Chapter 13, halogenated aromatic hydrocarbons, including polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs), are toxicants of much public and scientific concern because of their widespread distribution in the environment and the potential for human exposure from numerous sources. As previously noted, examples of the sources include combustion processes, contaminated pesticides, and industrial accidents or uncontrolled environmental discharge. Humans may be exposed to these toxicants by such routes as the inhalation of vapors, aerosols, or respirable particles, the ingestion of contaminated milk, meat, fish, water, and vegetation, and the dermal absorption of contaminants in soil or from pesticides.

PCDDs and some other halogenated aromatic hydrocarbons have been shown to be carcinogenic in rats and mice.[16,17] Hepatocellular carcinomas were produced in both species following dioxin exposure, but only female rats developed squamous cell carcinomas of the lung, nasal turbinates, and hard palate.[17] Ingestion of 0.1 μg/kg per day in the diet resulted in an increased incidence of these tumors. In addition to hepatocellular carcinomas, follicular cell adenomas of the thyroid were significantly increased in male rats and female mice.[17] Other studies also reported a dose–dependent increase in tumors of estrogen-sensitive organs, including breast, uterus, and pituitary.[18] This suggests that PCDDs may be acting through a hormone-like mechanism. Most evidence suggests that the carcinogenic activity of PCDDs results from a receptor-mediated, nongenotoxic, promotional mechanism.[19,20]

PCBs are carcinogenic in rodents, producing hepatocellular carcinoma in rats and hepatomas in mice.[20] The production of liver tumors in these species is related to the degree of chlorination of the PCB mixture.

The halogenated aromatic hydrocarbons are believed to produce their toxic effects through a common mechanism of action.[21] These compounds are able to induce hepatic cytochrome P450 microsomal enzyme systems, especially aryl hydrocarbon hydroxylase (AHH) activity. It is generally accepted that the toxic activity of these chemicals is related to their interaction with a cellular receptor, the *Ah* receptor, which regulates the synthesis of a number of cellular

Table 16.4 Chemicals, Groups of Chemicals, Complex Mixtures and Industrial Processes Causally Associated With Cancer in Humans

4-Aminobiphenyl	Coal-tar pitch
Analgesic mixtures containing phenacetin	Conjugated estrogens
Arsenic and certain arsenic compounds	Cyclophosphamide
Asbestos	Diethylstilbestrol
Azathioprine	Melphalan
Benzene	Methoxsalen with u.v. A therapy
Benzidine	Mineral oils (some)
Betel quid with tobacco	Mustard gas
N,N-Bis(2-chloroethyl)-2-naphthylamine	2-Naphthylamine
Bis(chloromethyl) ether and technical	Shale oils
Grade chloromethyl methyl ether	1,4-Butanediol dimethylsulfonate
Certain combined chemotherapy for products	Smokeless tobacco
Chlorambucil	Soots
Chromium and certain chromium compounds	Tobacco smoke
Coal tar	
Auramine manufacture	Isopropyl alcohol
Boot and shoe manufacture and repair	Nickel refining
Coal gasification (older processes)	Rubber
Coke production (certain exposures)	Treosulphan industry (certain occupations)
Furniture manufacture (wood dusts)	
Underground hematite mining (with exposures to radon)	

Source: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, *Supplement No. 4* (Updated).

proteins. As discussed in Chapters 13 and 14, the receptor is an intracellular protein that binds to these biphenolic compounds based on stereo-specific characteristics. Although the exact mechanism involved in the toxicity is not known, it is thought that the toxicity results from interference with the control of structural genes for several proteins through the binding of the *Ah* receptor (see Figure 13.11).

16.7 DNA REPAIR

As noted earlier, DNA is damaged by a variety of chemical and physical agents. Bases can be altered or lost, phosphodiester bonds in the backbone can be broken, and strands can become covalently crosslinked. These lesions are produced by ionizing radiation, UV light, and a variety of chemicals. It is important to note that much of the damage sustained by DNA can be repaired because genetic information is stored in both strands of the double helix, therefore information lost by one strand can be retrieved from the other.[22]

A variety of enzymatic processes function to repair damaged DNA, protecting cells from the potentially mutagenic and lethal effects of chemical agent- and oxygen-induced damage. In mammalian systems, there are two basic types of damage responses: repair mechanisms and tolerance mechanisms.[4] The repair mechanisms involve removal of the DNA damage, while the tolerance mechanisms circumvent the damage without fixing it. A number of enzyme systems exist, facilitating the repair. Four main steps are identified in the repair process in which enzyme systems are involved:

1. Recognition of the lesion by endonucleases.
2. Removal of the damaged portion by exonuclease.
3. Replacement of the damaged section of DNA by polymerases.
4. Rejoining the uninjured parts by ligases.

Although the repair process is quite efficient, errors may occur. Some of the errors may be expressed as visible chromosomal abnormalities, such as breaks, deletions, translocations, ring chromosomes, and sister chromatid exchanges. It is also considered likely that faulty DNA repair may lead to some mutations and cancer.

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16.9 REVIEW QUESTIONS

1. What is cancer?
2. What are the two most important causes of cancer?
3. What are the three stages of carcinogenesis? Which of these is(are) considered reversible?
4. What is the fundamental principle underlying the many theories about the causes of cancer?
5. What is the main cause of skin cancer?
6. How does short-wave UV radiation affect DNA?
7. Which one is most harmful, UV-A, UV-B, or UV-C?
8. List five metals that can induce cancer.
9. What is metastasis? How does it occur?
10. What are procarcinogens?
11. In what way does the superoxide free radical affect cancer?
12. How does vinyl chloride become a carcinogen?
13. What are nitroso compounds? Explain the way in which nitroso compounds can induce carcinogenesis.
14. Explain how BaP can be converted into its ultimate carcinogen.
15. How may DDE be related to carcinogenesis?
16. Explain the suggested mechanism by which dioxins act as a carcinogens.
17. Explain the mechanism that mammalian systems possess for repairing DNA damage.

Chapter 17

Ecological Risk Assessment

17.1 INTRODUCTION

Ecological risk assessment is the process that evaluates the potential adverse effects that human activities have on the plants and animals that make up ecosystems.[1] Ecological risk assessments also consider changes caused by human activities that alter important features of ecological systems, such as lakes, streams, forests, or watersheds. Anthropogenic changes may include, for example, the introduction of a new chemical, such as a pesticide, to a wheat field, or the alteration of a landscape that results from draining or filling a wetland.

Scientists often assess how much damage certain human actions may have on the plants or animals in an area in question. The risk assessment process provides a way to develop, organize and present scientific information so that it is relevant to environmental decision-making. Ecological risks may be local, such as a hazardous waste site; they may be regional, such as the Pacific Northwest regions of the U.S., or a certain section of the Mississippi River; or they may be global, such as emission of greenhouse gases, atmospheric transport of particulates, or global warming.

The early 1980s witnessed both the emergence of risk assessment as a regulatory paradigm and the first widespread use of ecological impact assessments to influence regulatory and policy decisions. The use of ecological information for decision-making has expanded slowly through the 1980s, as shown by the regulation of diazinon based on its impacts on birds, and action taken to tackle acid deposition in lakes.[1] In the middle to late 1980s, tools and methods for conducting ecological risk assessments began to be standardized, with the publication of several documents by U.S. Government agencies, such as the National Research Council and the Environmental Protection Agency (EPA).[2,3] After nearly two decades of effort and experiences, ecological risk assessment has become widely known as an important management tool for many Government officials and environmental scientists.

This chapter presents an introduction to the subject by summarizing several key points from available documents. In addition, a case study based on the EPA's *Guidelines for Ecological Risk Assessment*, published in 1998, is presented in Appendix 4 for reference.

17.2 BASIC COMPONENTS OF RISK ASSESSMENT

It is useful to first become familiar with several important terms commonly used in a risk assessment. These are shown below, with brief definitions:

- *Risk* – the probability of an adverse outcome; a combination of exposure and effects expressed as probability.
- *Stressor* – any physical, chemical, or biological entity that can induce an adverse response on a biological system (synonymous with *agent*).
- *Exposure* – the contact or co-occurrence of a *stressor* with a *receptor*.
- *Hazard* – used in the U.S. and Canada to refer to intrinsic toxic properties, while internationally it refers to the probability of an adverse outcome.
- *Receptor* – the ecological entity exposed to the *stressor*.
- *Uncertainty* – a lack of confidence in the prediction that may be due to natural variability in environmental processes, errors in conducting an assessment, or incomplete knowledge about certain specific aspects of exposure.
- *Risk assessor* – an individual or team with the appropriate training or range of expertise necessary to conduct a risk assessment.
- *Risk manager* – an individual, team, or organization, that can make decisions or take action concerning alternatives for addressing risks (In some cases, *risk managers* may include interested parties or stakeholders.)

17.3 USE OF ECOLOGICAL RISK ASSESSMENT

The ecological risk assessment process is used to systematically evaluate and organize data, information, assumptions, and uncertainties in order to help understand and predict the relationships between stressors and ecological effects in a way that is useful for environmental decision-making. Assessment may involve physical, chemical, or biological stressors, and may include one stressor or many stressors.

As noted, an ecological risk assessment evaluates the potential adverse effects that human activities have on the plants and animals that make up ecosystems. The risk assessment process provides a way to develop, organize and present scientific information so that it is relevant to environmental decisions. When conducted for a particular place, such as a watershed, the ecological risk assessment process can be used to identify vulnerable and valued resources, prioritize data-collection activities, and link human activities with their potential effects. Risk assessments can also provide a focal point for cooperation between local communities and state and federal government agencies.

Ecological risk assessment is one input into environmental management decisions. Other inputs include stakeholder concerns, availability of technical solutions, benefits, equity, costs, legal mandates, and political issues. Risk assessment results provide a basis for comparing different management

options, enabling decision-makers and the public to make better-informed decisions about the management of ecological resources.[1]

Ecological risk assessments can also be used to predict the likelihood of future adverse effects (prospective) or evaluate the likelihood that effects are caused by past exposure to stressors (retrospective). In many cases, both approaches are included in a single risk assessment.

17.4 IMPORTANCE OF ECOLOGICAL RISK ASSESSMENT

A great deal of research conducted in the field is geared toward the determination of the risk of producing a new product or releasing chemicals, such as a pesticide or an industrial effluent, to ecosystems. As noted previously, ecological risk assessments are tools that decision-makers can use to help them identify and, hopefully, reduce uncertainty throughout the decision-making process.

Ecosystem assessments follow general concepts, as shown in Figure 17.1, but there is no predetermined set of rules for undertaking an assessment. The general concepts include acknowledgment of stakeholders and their questions, development of situational analyses, identification of limits and trade-offs,

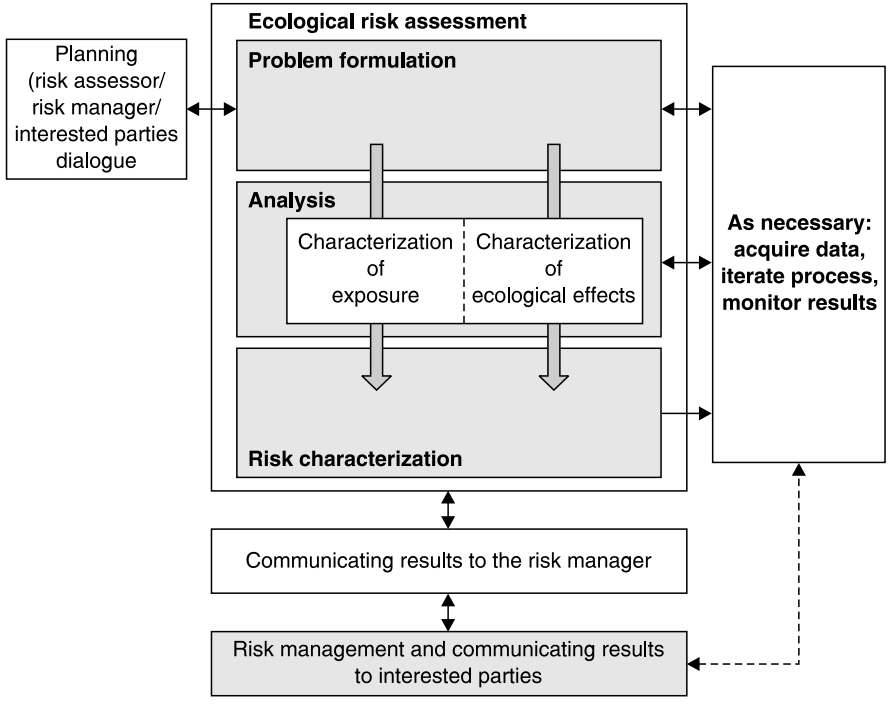
Ecological risk assessment
Planning (risk assessor/ risk manager/ interested parties dialogue
Problem formulation
Analysis
Characterization of exposure
Characterization of ecological effects
Risk characterization
As necessary: acquire data, iterate process, monitor results
Communicating results to the risk manager
Risk management and communicating results to interested parties

Figure 17.1 The framework for ecological risk assessment.
Source: adapted from EPA, *Framework for Ecological Risk Assessment*, Washington, 1992.

development of an understanding of future conditions, and assessment of risk for issues of concern. The primary reason for conducting ecosystem assessments is to provide a framework for decision makers and stakeholders to help them understand and evaluate the consequences of actions concerning regulation or allocation of natural resources within the larger social and ecological context.[1]

The endpoints of risk assessment are often set by societal perceptions and values. Although scientific process may be used in collecting information for the assignment of risks, unless a testable hypothesis can be formulated, the scientific method is not being applied. For example, a course of action that has the least ecological risk may be too expensive or not technologically feasible. Therefore, while an ecological risk assessment provides critical information to risk managers, it is only one part of the whole environmental decision-making process.

Environmental toxicology and risk assessment are closely related. Environmental toxicology, as with any branch of science, attempts to answer specific questions. In this case, the question may be primarily focused on how a particular xenobiotic (or xenobiotics) interacts with the components of an ecological system. The background knowledge obtained from the study of environmental toxicology can serve as an important basis for significantly contributing to the process of risk assessment.

17.5 FRAMEWORKS FOR ECOLOGICAL RISK ASSESSMENT

The ecological risk assessment process is based on two major elements: characterization of effects, and characterization of exposure. These elements were proposed over the past 10 years, one of them based on a National Academy of Sciences report detailing risk assessment for federal agencies.

As shown in Figure 17.1, the framework is composed of three principal elements or phases: problem formulation, analysis, and risk characterization. Problem formulation involves a clear definition of the specific problem under consideration. This phase can ultimately influence the scientific validity and policy related to the risk assessment process. The second phase in the process, analysis, is subdivided into characterization of potential or existing exposure to stressors, and characterization of ecological effects. The last step, risk characterization, consists of integration and evaluation of exposure and effects information.

17.5.1 Problem Formulation

In problem formulation, the purpose for the assessment is stated, the problem is defined, and a plan for analyzing and characterizing risk is determined. The process is made up of several elements: discussion between the risk assessor and risk manager, stressor characteristics, identification of the ecosystem potentially at risk, ecological effects, endpoint selection, conceptual modeling, and

input from data acquisition, verification, and monitoring. The initial work in problem formulation includes the integration of available information on sources, stressors, effects, and ecosystem and receptor characteristics. The information obtained contributes to the generation of two products: assessment endpoints and conceptual models. Either product may be generated first (and the order depends on the type of risk assessment), but both are needed to complete an analysis plan, the final product of problem formulation.

The process may be initiated by various causes. For example, a request for the introduction of a new material into the environment, or for the determination of clean-up or land-use options for a contaminated site.

A critical aspect for the problem formulation process is the emphasis that is placed on the importance of discussions between the risk assessor and the risk manager, the importance of acquisition of new data, and verification of the risk assessment and monitoring. The discussion between the risk assessor and risk manager of societal goals and scientific reality helps to set the boundaries for the scope of the risk assessment. The interaction between these individuals can help to consolidate the goals into definable components of a risk assessment.

17.5.2 Analysis

Analysis is directed by the outcome of problem formulation. As indicated previously, analysis consists of two phases: characterization of exposure and characterization of ecological effects (Figure 17.1). In characterization of exposure, the data resulting from the problem formulation are evaluated to determine how exposure to stressors is likely to occur. The strength and limitations of data concerning exposure, effects, and ecosystem and receptor characteristics are evaluated. As mentioned previously, exposure is the interaction of stressors with receptors. In the assessment of hazard due to exposure, details of the biological effects of the stressor under examination are assessed. Measures of exposure can include concentrations of contaminants, such as tissue levels of DDT in habitat, or physical changes, such as body weight.

The exposure potential of critical biological components to the material is assessed as part of an exposure characterization. Risk assessment requires qualitative information about the strength of the evidence of the exposure and the nature of the outcomes, as well as quantitative assessment of the exposures, host susceptibility factors, and potential magnitude of the risk, and then a description of the uncertainties in the estimates and conclusions.

Stressor characteristics form an important aspect of the risk assessment process. Stressors can be physical, chemical, or biological in nature. Biological stressors could include the introduction of a new species or the application of a specific fertilizer to farming. Physical stressors may include changes in temperature or geological processes. Examples of chemical stressors may include such materials as pesticides or industrial effluents. Chemical stressors may include intensity, such as dose or concentrations of chemical agents, duration, timing, or frequency of actions.

The above step is followed by characterization of ecological effects, i.e., determination of the potential and type of ecological effects that can be anticipated. Myriad interactions exist between the stressor and the ecological system and each should be considered. Examples of interactions include acute and chronic toxicity, bioaccumulation, biodegradation, biotransformation, predator–prey interactions, community resilience, and evolutionary impacts. Available data are analyzed to characterize the nature of potential or actual exposure and the ecological responses under the defined circumstances.

Ecosystems potentially at risk may be more difficult to characterize. Ecosystems consist of a large number of biotic and abiotic characteristics, which must be considered in the process. For instance, sediments have both biotic and abiotic components that can dramatically affect contaminant availability. Geographic relationship to adjacent systems is another key characteristic, influencing species migration and therefore recovery rates from the influence of stressors. Additionally, size of the ecosystem is also an important variable, affecting the number of species and the complexity of the system itself.

17.5.3 Risk Characterization

The third and final phase of the risk assessment process is risk characterization (Figure 17.1). This involves integration and evaluation of exposure and effects information. The overall process is to combine the ecological effects with the environmental concentrations to provide the likelihood of effects in the presence of the stressor within the system. It is important to point out that a stressor poses no risk to an environment unless it involves exposure. Virtually all materials have some characteristic biological effect; however, unless a sufficient amount of the stressor interacts with a biological system, no effects can occur. Risk is a combination of exposure and resultant effects expressed as a probability. Integrating exposure and effects information leads to an estimation of risk, the likelihood that adverse effects will result from exposure.

Approaches for evaluating exposure and effects include, for example, measuring chemical releases, predicting the environmental fate and effects of chemicals (possibly even before they are manufactured), and testing the effects of these chemicals in a laboratory. Exposure and effects must be considered together because they are both important in assessing risk. When the potential for exposure and effects are low, the risk will be low. When both are high, the risk will be high. Whatever the approach, the goal is to use all available information to characterize exposure and effects and to integrate them into an understanding of ecological risks.[4]

The integration of exposure with toxicity needs to be conducted with caution. As noted in the previous chapters, environmental toxicology deals with a variety of effects at different levels of biological organization. A widely used method for estimating risk is the quotient method.[4] This method is based

on simple division of the expected environmental concentration by the concentration producing an unacceptable effect, i.e., hazard:

$$\text{Quotient} = \frac{\text{Expected environmental concentration}}{\text{Concentration producing an unacceptable effect}}$$

The resultant quotient is generally judged by the criteria shown below:

Quotient	*Risk*
>1	Potent or high risk
~ 1	Potential risk
<1	Low risk

As indicated previously, because of the complexity of natural systems, risk assessment will include some degree of uncertainty. Although it is possible to reduce some components of uncertainty by collecting additional data, it may only be possible to estimate other components due to their inherent variability, e.g. weather variations. While it is important for risk managers to understand the impact of natural variability and uncertainty on the conclusions of the risk assessment, making a risk management decision does not require the absence of uncertainty. In fact, attempts are normally made to quantify and communicate uncertainty when conducting and reporting ecological risk assessment so that the best decisions can be made given the available knowledge.[5]

Although analysis and risk characterization are shown as separate phases, some models may combine the analysis of exposure and effects data with the integration of these data that occurs in risk characterization.

17.6 USEFULNESS OF ECOLOGICAL RISK ASSESSMENT PREDICTIONS

Although there are various sources of uncertainty in ecological risk assessment, it is possible to predict many effects with confidence. Even when uncertainties are high, risk assessments based on proper scientific review and consensus provide the best summary of the state of knowledge.

Ecological risk assessment results are most useful when risk managers clearly communicate the risks and decisions to the public. An ecological risk assessment should

- summarize results so that the public can understand them
- distinguish scientific conclusions from policy judgments
- describe major differences of opinion on scientific conclusions that readers can draw from the data
- explain major assumptions and uncertainties

Because of the complexity and variability of nature, the initial scoping phase of an ecological risk assessment (problem formulation) is critical for providing a focus for the assessment. However, ecological risk assessments need not be complex or lengthy, they only need to define the risks with the degree of certainty required to support a risk management decision.[5]

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17.8 REVIEW QUESTIONS

1. What are ecological risk assessments?
2. Define the terms ''exposure,'' ''stressor'' and ''hazard.''
3. Define ''problem formulation.''
4. What is an endpoint?
5. What is the quotient method of estimating risk?
6. What are the ways to determine exposure?
7. What is the goal of exposure analysis?
8. Describe the importance of communications between risk assessor and risk manager.

Appendix 1

Glossary

Abscission. Process by which a leaf or other part is separated from the plant.

Acetylcholine (ACh). Chemical transmitter of nerve and nerve–muscle impulses in animals.

Acetylcholinesterase (AChE). An enzyme of the body necessary for proper nerve function, which is inhibited or damaged by organophosphate or carbamate insecticides taken into the body by any route.

Acute toxicity. The toxicity of a material determined at the end of 24 hours to cause injury or death from single dose or exposure.

Adsorption. Chemical and/or physical attraction of a substance to a surface. Refers to gases, dissolved substances, or liquids on the surface of solids or liquids.

Aerosol. Colloidal suspension of solids or liquids in air.

Alkylating agent. Highly active compounds that replace hydrogen atoms with alkyl groups, usually in cells undergoing division.

Alumina. Aluminum oxide, Al_2O_3.

Aminotransferase. An enzyme that catalyzes transamination.

Anabolism. Constructive metabolism – opposite of *catabolism*.

Aneuploidy. Chromosomal changes that involve only single chromosomes within a set.

Antagonism. Decreased activity arising from the effect of one chemical or another (opposite of *synergism*).

Anthropogenic. Induced or altered by the presence and activities of humans.

Apoenzyme. The protein without prosthetic group (in an enzymatic system).

Arsenism. A disease caused by arsenic poisoning.

Berylliosis. Chronic beryllium disease.

Bilirubin. A reddish yellow crystalline pigment occurring in bile, blood, urine, and gallstones.

Biomagnification. The increase in concentration of a pollutant in animals as related to their position in a food chain, usually referring to the persistent, *organochlorine insecticides* and their metabolites.

Biotransformation. Metabolic conversion of a toxicant in the body.

Broad-spectrum insecticide. Nonselective, having about the same toxicity to most insects.

Bronchiolitis. Chronic inflammation of bronchioles.

Cachexia. A general physical wasting and malnutrition caused by a chronic disease.

Calcination. The action or process of calcining (heating under oxidizing conditions or converting to a powder by heating).

Catabolism. Destructive metabolism involving release of energy – opposite of *anabolism*.

Carbamate insecticide. One of a class of insecticides derived from carbamic acid.

Carcinogen. A substance that causes cancer in animal tissue.

Carcinogenic. Producing or tending to produce cancer.

Carcinogenesis. The development of cancer.

Carrier. An inert material that serves as a diluent or vehicle for an active ingredient or toxicant.

Chelating agent. Certain organic chemicals (e.g., ethylenediaminetetraacetic acid, EDTA) that combine with metal to form soluble chelates and prevent conversion to insoluble compounds.

Chelation. A process wherein atoms of a metal in solution are sequestered by ring-shaped chemical species.

Chlososis. A diseased condition of chlorophyll-bearing plants manifested as yellowing or blanching of the normally green parts (leaves).

Chronic bronchitis. Bronchitis is inflammation of the bronchi, resulting in a persistent cough that produces considerable quantities of sputum. When the condition is persistent over a long period and recurring over several years, it is referred to as chronic bronchitis.

Chronic toxicity. The toxicity of a material determined beyond 24 hours and usually after several weeks of exposure.

Ciliagenesis. Production of cilia.

Cirrhosis. A chronic progressive disease of the liver that is characterized by an excessive formation of connective tissue followed by hardening.

Congenital. Acquired during development in the uterus and not through heredity.

Cryolite. Sodium aluminum fluoride (Na_3AlF_6).

Dealkylation. The process of removing an alkyl group from (a compound).

Deamination. The process of removing an amino group from (a compound).

Defoliant. A chemical that initiates *abscission*.

Demethylation. Removal of methyl from (a compound, such as a DNA base).

Denaturation. The process of denaturing — used especially for proteins.

Denature. To deprive of natural qualities or characteristics

Depurination. Removal of a purine base.

Dermal toxicity. Toxicity of a material as tested on the skin, usually on the shaved belly of a rabbit; the property of a *pesticide* to poison an animal or human when absorbed through the skin.

Detoxify. To make an active ingredient in a *pesticide* or other poisonous chemical harmless and incapable of being toxic to plants and animals.

Dimerization. Formation of a dimer (e.g., from two DNA bases such as thymine).

Dyspnea. Short of breath.

Diluent. A diluting agent.

Dose, dosage. The amount of toxicant given or applied per unit of plant, animal, or surface. Same as rate.

EC_{50}. The median effective concentration (ppm or ppb) of the toxicant in the environment (usually water) that produces a designated effect in 50% of the test organisms exposed.

ED_{50}. The median effective dose, expressed as mg/kg of body weight, which produces a designated effect in 50% of the test organisms exposed.

Edema. An abnormal accumulation of serous fluid in connective tissue causing puffy swelling.

Emaciation Wasted condition of the body.

Emphysema. A condition of the lung marked by distention, progressive loss of elasticity, and eventual rupture of the alveoli and accompanied by labored breathing and a husky cough.

Endocrine disrupter. An exogenous agent capable of disrupting the normal function of endocrine system.

Endogenous. Arising from internal structural or functional causes.

Epidemiology. The science that deals with the incidence, distribution, and control of disease in a population.

Epigastric pain. Pain over the abdomen.

Erythrocytosis. See *polycythemia*.

Estrogenicity. Promotion of estrus.

Eucaryotic. Having a visibly evident nucleus.

Euploidy. A chromosomal change involving entire sets of chromosomes.

Exogenous. Arising from outside.

Exostotic. Having a bony outgrowth from a bone or the root of a tooth.

Exposure. The contact or co-occurrence of a *stressor* with a *receptor*.

Extractant. A solvent used for extracting organic compounds from plant or animal tissues.

Fibrosis. A condition characterized by deposition of excessive amounts of collagen.

Fluorosis. An abnormal or poisoned condition caused by fluorine or its compounds.

Fluorspar. Calcium fluoride, CaF_2.

Fluoraptite. Calcium fluorophosphate, $Ca_{10}F_2C(PO_4)_6$.

Food chain. Sequence of species within a community, each member of which serves as food for the species next higher in the chain.

Fumigant. A volatile material that forms vapors that destroy insects, pathogens, and other pests.

Fungicide. A chemical that kills fungi.

Glucosuria. Occurrence of glucose in the urine.

Glycogenolysis. Breakdown of glycogen into glucose in the liver and muscle.

Hazard. As used in ecological risk assessment, hazard refers to the potential adverse ecological effects of a *stressor*.

Hematopoiesis. Formation of blood or of blood cells within the living body.

Hepatoma. A tumor of the liver that is usually malignant.

Hemolysis. Liberation of hemoglobin from red blood cells.

Holoenzyme. Catalytically active complex of protein and prosthetic group.

Homeostasis. A tendency toward maintenance of a relatively stable internal environment in the bodies of higher animals (through a series of interacting physiological processes).

Hormone. A product of living cells that circulates in animal or plant fluids and that produces a specific effect on cell activity remote from its point of origin.

Hydrolysis. Chemical process of breakdown or decomposition involving a splitting of the molecule and addition of a water molecule.

Hyperostosis. Excessive formation of bone tissue especially in the skull.

Hyperplasia. Abnormal increase in the number of cells of a tissue.

Hypertrophy. Abnormal increase in the size of cells of a tissue.

Hyperuricemia. Increase in the uric acid concentrations in blood.

Hypoplasia. A thin enamel.

Hypoproteinemia. Low protein levels of blood.

Hypospadias. An abnormality of the penis in which the urethra opens on the under surface.

Hypoxia. A deficiency of oxygen reaching the tissues of the body.

Imposex. A condition in which females develop parts of the male reproductive system.

Isoenzymes (or isozymes). Enzymes that catalyze the same reaction but migrate differently on electrophoresis.

Inhalation. Exposure of test animals either to vapor or dust for a predetermined time.

Intragenic deletion. An extensive deletion of a gene so that the information material of that gene is essentially lost.

Invertase. The enzyme that breaks down sucrose into glucose and fructose.

Itai-itai-byo. Ouch-ouch-disease, caused by cadmium poisoning.

Lacrimation. Secretion of tears.

LC_{50}. The median lethal concentration, the concentration that kills 50% of the test organisms, expressed as mg or ml (if liquid) per animal. It is also the concentration expressed as parts per million (ppm) or parts per billion (ppb) in the environment (usually water) that kills 50% of the test organism exposed.

LD_{50}. A lethal dose for 50% of the test organisms. The dose of toxicant producing 50% t mortality in a population. A value used in presenting mammalian toxicity, usually oral toxicity, expressed as mg of toxicant per kg of body weight (mg/kg).

Leachate. The liquid that has percolated through soil or other medium.

Leukocytosis. An increase in the number of leukocytes in the circulating blood.

Lipolysis. Breakdown of fats.

Lipophilicity. Having strong affinity for fats.

Lowest observed adverse effect level (LOAEL). The lowest level of a *stressor* evaluated in a test that causes statistically significant differences from the controls.

Lumina. Plural form of lumen, the cavity of passageway of a tubular organ.

Lysis. A process of disintegration or dissolution.

Lysozyme. A basic protein that is present in egg white and in biological secretions; functions as a mucolytic enzyme and is capable of attacking the capsules of various bacteria.

Mercurial. A chemical compound that contains mercury.

Mesocosm. A large *microcosm*; usually, but not always, involves more trophic levels and generally a greater complexity than a microcosm toxicity test.

Metabolism. The sum of all chemical reactions that occur within a living cell.

Metallothionein. Low-molecular-weight, nonenzymatic proteins with unique amino acid composition.

Metastasis. Spread of malignant cells from the primary site to other sites of the body.

Methemoglobin. A soluble brown crystalline basic pigment that is formed from blood, hemoglobin, or oxyhemoglobin by oxidation.

Microcephaly. A condition of unusual smallness of head usually associated with mental defects.

Microcosm. A type of multispecies toxicity test; also called small cosmos toxicity test.

Microphthalmia. Abnormal smallness of the eye.

Myopathy. Disorder of muscle tissue or muscles.

Morbidity. The incidence of disease.

Morphogenesis. The formation and differentiation of tissues or organs.

Mutagen. An agent that tends to increase the occurrence or extent of mutation.

Necrosis. Death of tissue, plant or animal.

Neoplasm. A new growth of animal or plant tissue resembling more or less the tissue from which it arises but serving no physiologic function, and being benign, potentially malignant.

Nephrosis. Noninflammatory degeneration of the kidneys, chiefly affecting the tubules.

Nephrotoxin. A toxicant that can poison the kidney.

Nitrosation. The process of converting into a nitroso compound.

No observed adverse effect level (NOAEL). The highest level of a *stressor* evaluated in a test that does not cause statistically significant differences from the controls.

Oncogenic. The property to produce tumors (not necessarily cancerous) in tissues.

Organochlorine insecticide. One of the many chlorinated insecticides, e.g., DDT, dieldrin, BHC, chlordane, etc.

Organophosphate. Class of insecticides (also one or two herbicides and fungicides) derived from phosphoric acid esters.

Osteomalacia. A disease of the bones characterized by softening, affecting adults of man and domestic animals.

Paraesthesia. An abnormal sensation, such as prinkling, itching, etc.

Pathogen. Any disease-producing organism or virus.

Peroxidation. The process of forming a peroxide.

Persistence (for an insecticide). The quality of an insecticide to persist as an effective residue due to its low volatility and chemical stability, e.g., certain *organochlorine insecticides*.

Pesticide. An "economic poison" defined in most state and federal laws as any substance used for controlling, preventing, destroying, repelling, or mitigating any pest. Includes fungicides, herbicides, insecticides, nematicides, rodenticides, and defoliants.

Pharyngitis. Inflammation of pharynx.

Phytochelatin. A class of sulfur-rich polypeptide that occurs in plants and can form a complex and thus neutralize a toxic metal such as cadmium.

Phytotoxic. Injurious to plants.

Pica. Craving for and eating of unnatural substances.

Pneumonitis. A disease characterized by inflammation of the lungs.

Pneumonoconiosis. A disease of the lung caused by habitual inhalation of irritant mineral or metallic particles.

Point mutation. A kind of mutation involving displacement of a nucleic acid base with another or insertion or deletion of a nucleoside within a polynucleotide sequence of a gene.

Polycythemia. A condition marked by an abnormal increase in the number of circulating red blood cells.

Polymorphism. Capability of assuming different forms.

Potentiation. The action or process of causing an increase in physiological activity.

Problem formulation. The initial stage of an ecological risk assessment where the purpose of the assessment is articulated, assessment endpoints and a conceptual model are developed, and a plan for analyzing and characterizing risk is determined.

Procarcinogen. A *carcinogen* that requires biological activation.

Proliferation. Rapid and repeated production of new parts or of buds or offspring.

Proteinuria. Occurrence of proteins in the urine.

Pyrolysis. Chemical decomposition or other chemical change brought about by the action of heat regardless of the temperature involved.

Receptor. The ecological entity exposed to a *stressor*.

Remediation. The act or process of remedying, usually referring to an application that serves to restore the health of an affected or contaminated site.

Resistance (insecticide). Natural or genetic ability of an organism to tolerate the poisonous effects of a *toxicant*.

Risk analysis. The process that includes both risk assessment and risk management.

Risk assessor. An individual or team with the appropriate training or range of expertise necessary to conduct an ecological risk assessment.

Risk manager. An individual, team, or organization that can make decisions or take action concerning alternatives for addressing risks. In some

situations, risk managers may include a wide range of interested parties or stakeholders.

Rodenticide. *Pesticide* applied as a bait, dust, or fumigant to destroy or repel rodents and other animals, such as moles and rabbits.

Silicatosis. A disease caused by habitual inhalation of silicates.

Silicosis. A disease caused by habitual inhalation of silica.

Sinusitis. Inflammation of sinus.

Source. An entity or action that releases to the environment or imposes on the environment a chemical, physical, or biological *stressor*.

Splenomegaly. Enlargement of the spleen.

Stressor. Any physical, chemical, or biological entity that can induce an adverse response (synonymous with agent).

Sural. Relating to branches of the popliteral artery or vein that ramify in the calf of the leg.

Synergism. Increased activity resulting from the effect of one chemical on another.

Systemic. Compound that is absorbed and translocated throughout the plant or animal.

Target. The plants, animals, structures, areas, or pests to be treated with a *pesticide* application; or the plant/animal structure or organ where a toxicant exerts its toxic action.

Teratogen. An agent that causes teratogenesis.

Teratogenic. Relating to teratogenesis.

Teratogenesis. Production of monstrous growths or fetuses.

Thyroxine. A crystalline iodine-containing substance that is the chief active principal of the thyroid gland.

TLV. Threshold limit value.

TLV-TWA. Time-weighted average threshold limit value. The maximum time-weighted average concentration to which a healthy worker may be exposed, for a normal 40-hour work week up to 8 hours a day over a working lifetime (40 to 50 years), without becoming ill.

Tolerance. Amount of *toxicant*/*pesticide* residue permitted by federal regulation to remain on or in a crop (expressed as ppm).

Tolerant. Capable of withstanding effects.

Toxicant. A poisonous substance such as the active ingredient in *pesticide* formulations that can injure or kill plants, animals, or microorganisms.

Toxin. A naturally occurring poison produced by plants, animals, or microorganism; for example, the poison produced by the black widow spider, the venom produced by snakes, and the botulism toxin.

Tracheobronchitis. Inflammation of the trachea and bronchi.

Transplacental carcinogen. A chemical, which when given to the mother, causes cancer in her daughter.

Vesicle. A plant or animal structure having the general form of a membranous cavity.

Xenobiotics. Toxicants found in the environment.

Appendix 2

PCB Nomenclature

BZ&IUPAC#	IUPAC Name	CASRN
1	2-Chlorobiphenyl	2051-60-7
2	3-Chlorobiphenyl	2051-61-8
3	4-Chlorobiphenyl	2051-62-9
4	2,2′-Dichlorobiphenyl	13029-08-8
5	2,3-Dichlorobiphenyl	16605-91-7
6	2,3′-Dichlorobiphenyl	25569-80-6
7	2,4-Dichlorobiphenyl	33284-50-3
8	2,4′-Dichlorobiphenyl	34883-43-7
9	2,5-Dichlorobiphenyl	34883-39-1
10	2,6-Dichlorobiphenyl	33146-45-1
11	3,3′-Dichlorobiphenyl	2050-67-1
12	3,4-Dichlorobiphenyl	2974-92-7
13	3,4′-Dichlorobiphenyl	2974-90-5
14	3,5-Dichlorobiphenyl	34883-41-5
15	4,4′-Dichlorobiphenyl	2050-68-2
16	2,2′,3-Trichlorobiphenyl	38444-78-9
17	2,2′,4-Trichlorobiphenyl	37680-66-3
18	2,2′,5-Trichlorobiphenyl	37680-65-2
19	2,2′,6-Trichlorobiphenyl	38444-73-4
20	2,3,3′-Trichlorobiphenyl	38444-84-7
21	2,3,4-Trichlorobiphenyl	55702-46-0
22	2,3,4′-Trichlorobiphenyl	38444-85-8
23	2,3,5-Trichlorobiphenyl	55720-44-0
24	2,3,6-Trichlorobiphenyl	55702-45-9
25	2,3′,4-Trichlorobiphenyl	55712-37-3
26	2,3′,5-Trichlorobiphenyl	38444-81-4
27	2,3′,6-Trichlorobiphenyl	38444-76-7
28	2,4,4′-Trichlorobiphenyl	7012-37-5
29	2,4,5-Trichlorobiphenyl	15862-07-4
30	2,4,6-Trichlorobiphenyl	35693-92-6
31	2,4′,5-Trichlorobiphenyl	16606-02-3
32	2,4′,6-Trichlorobiphenyl	38444-77-8
33	2,3′,4′-Trichlorobiphenyl	38444-86-9
34	2,3′,5′-Trichlorobiphenyl	37680-68-5
35	3,3′,4-Trichlorobiphenyl	37680-69-6
36	3,3′,5-Trichlorobiphenyl	38444-87-0
37	3,4,4′-Trichlorobiphenyl	38444-90-5
38	3,4,5-Trichlorobiphenyl	53555-66-1
39	3,4′,5-Trichlorobiphenyl	38444-88-1
40	2,2′,3,3′-Tetrachlorobiphenyl	38444-93-8
41	2,2′,3,4-Tetrachlorobiphenyl	52663-59-9
42	2,2′,3,4′-Tetrachlorobiphenyl	36559-22-5
43	2,2′,3,5-Tetrachlorobiphenyl	70362-46-8
44	2,2′,3,5′-Tetrachlorobiphenyl	41464-39-5
45	2,2′,3,6-Tetrachlorobiphenyl	70362-45-7
46	2,2′,3,6′-Tetrachlorobiphenyl	41464-47-5
47	2,2′,4,4′-Tetrachlorobiphenyl	2437-79-8
48	2,2′,4,5-Tetrachlorobiphenyl	70362-47-9
49	2,2′,4,5′-Tetrachlorobiphenyl	41464-40-8

BZ&IUPAC#	IUPAC Name	CASRN
50	2,2′2,4,6-Tetrachlorobiphenyl	62796-65-0
51	2,2′,4,6′-Tetrachlorobiphenyl	68194-04-7
52	2,2′,5,5′-Tetrachlorobiphenyl	35693-99-3
53	2,2′,5,6′-Tetrachlorobiphenyl	41464-41-9
54	2,2′,6,6′-Tetrachlorobiphenyl	15968-05-5
55	2,3,3′,4-Tetrachlorobiphenyl	74338-24-2
56	2,3,3′,4′-Tetrachlorobiphenyl	41464-43-1
57	2,3,3′,5-Tetrachlorobiphenyl	70424-67-8
58	2,3,3′,5′-Tetrachlorobiphenyl	41464-49-7
59	2,3,3′,6-Tetrachlorobiphenyl	74472-33-6
60	2,3,4,4′-Tetrachlorobiphenyl	33025-41-1
61	2,3,4,5-Tetrachlorobiphenyl	33284-53-6
62	2,3,4,6-Tetrachlorobiphenyl	54230-22-7
63	2,3,4′,5-Tetrachlorobiphenyl	74472-34-7
64	2,3,4′,6-Tetrachlorobiphenyl	52663-58-8
65	2,3,5,6-Tetrachlorobiphenyl	33284-54-7
66	2,3′,4,4′-Tetrachlorobiphenyl	32598-10-0
67	2,3′,4,5-Tetrachlorobiphenyl	73575-53-8
68	2,3′,4,5′-Tetrachlorobiphenyl	73575-52-7
69	2,3′,4,6-Tetrachlorobiphenyl	60233-24-1
70	2,3′,4′,5-Tetrachlorobiphenyl	32598-11-1
71	2,3′,4′,6-Tetrachlorobiphenyl	41464-46-4
72	2,3′,5,5′-Tetrachlorobiphenyl	41464-42-0
73	2,3′,5′,6-Tetrachlorobiphenyl	74338-23-1
74	2,4,4′,5-Tetrachlorobiphenyl	32690-93-0
75	2,4,4′6-Tetrachlorobiphenyl	32598-12-2
76	2,3′,4′,5′-Tetrachlorobiphenyl	70362-48-0
77	3,3′,4,′-Tetrachlorobiphenyl	32598-13-3
78	3,3′,4,5-Tetrachlorobiphenyl	70362-49-1
79	3,3′,4,5′-Tetrachlorobiphenyl	41464-48-6
80	3,3′,5,5′-Tetrachlorobiphenyl	33284-52-5
81	3,4,4′,5-Tetrachlorobiphenyl	70362-50-4
82	2,2′,3,3′,4-Pentachlorobiphenyl	52663-62-4
83	2,2′,3,3′,5-Pentachlorobiphenyl	60145-20-2
84	2,2′,3,3′,6-Pentachlorobiphenyl	52663-60-2
85	2,2′,3,4,4′-Pentachlorobiphenyl	65510-45-4
86	2,2′,3,4,5-Pentachlorobiphenyl	55312-69-1
87	2,2′,3,4,5′-Pentachlorobiphenyl	38380-02-8
88	2,2′,3,4,6-Pentachlorobiphenyl	55215-17-3
89	2,2′,3,4,6′-Pentachlorobiphenyl	73575-57-2
90	2,2′,3,4′,5-Pentachlorobiphenyl	69194-07-0
91	2,2′,3,4′,6-Pentachlorobiphenyl	68194-05-8
92	2,2′,3,5,5′-Pentachlorobiphenyl	52663-61-3
93	2,2′,3,5,6-Pentachlorobiphenyl	73575-56-1
94	2,2′,3,5,6′-Pentachlorobiphenyl	73575-55-0
95	2,2′3,5′,6-Pentachlorobiphenyl	38379-99-6
96	2,2′,3,6,6′-Pentachlorobiphenyl	73575-54-9
97	2,2′,3,4′,5′-Pentachlorobiphenyl	41464-51-1
98	2,2′,3,4′,6′-Pentachlorobiphenyl	60233-25-2
99	2,2′,4,4′,5-Pentachlorobiphenyl	38380-01-7
100	2,2′,4,4′,6-Pentachlorobiphenyl	39485-83-1
101	2,2′,4,5,5′-Pentachlorobiphenyl	37680-73-2
102	2,2′,4,5,6′-Pentachlorobiphenyl	68194-06-9
103	2,2′,4,5′,6-Pentachlorobiphenyl	60145-21-3
104	2,2′,4,6,6′-Pentachlorobiphenyl	56558-16-8
105	2,3,3′,4,4′-Pentachlorobiphenyl	32598-14-4
106	2,3,3′,4,5-Pentachlorobiphenyl	70424-69-0
107	2,3,3′,4′,5-Pentachlorobiphenyl	70424-68-9

BZ&IUPAC#	IUPAC Name	CASRN
108	2,3,3′,4,5′-Pentachlorobiphenyl	70362-41-3
109	2,3,3′,4,6-Pentachlorobiphenyl	74427-35-8
110	2,3,3′,4′,6-Pentachlorobiphenyl	328380-03-9
111	2,3,3′,5,5′-Pentachlorobiphenyl	39635-32-0
112	2,3,3′,5,6-Pentachlorobiphenyl	74427-36-9
113	2,3,3′,5′,6-Pentachlorobiphenyl	68194-10-5
114	2,3,4,4′,5-Pentachlorobiphenyl	74427-37-0
115	2,3,4,4′,6-Pentachlorobiphenyl	74427-38-1
116	2,3,4,5,6-Pentachlorobiphenyl	18259-05-7
117	2,3,4′,5,6-Pentachlorobiphenyl	68194-11-6
118	2,3′,4,4′,5-Pentachlorobiphenyl	31508-00-6
119	2,3′,4,4′,6-Pentachlorobiphenyl	56558-17-9
120	2,3′,4,5,′-Pentachlorobiphenyl	68194-12-7
121	2,3′,4,5′,6-Pentachlorobiphenyl	56558-18-0
122	2,3,3′,4′,5′-Pentachlorobiphenyl	76842-07-4
123	2,3′,4,4′,5′-Pentachlorobiphenyl	65510-44-3
124	2,3′,4′,5,5′-Pentachlorobiphenyl	70424-70-3
125	2,3′,4′,5′,6-Pentachlorobiphenyl	74427-39-2
126	3,3′,4,4′,5-Pentachlorobiphenyl	57465-28-8
127	3,3′,4,5,5′-Pentachlorobiphenyl	39635-33-1
128	2,2′,3,3′,4,4′-Hexachlorobiphenyl	38380-07-3
129	2,2′,3,3′,4,5-Hexachlorobiphenyl	55215-18-4
130	2,2′,3,3′,4,5′-Hexachlorobiphenyl	52663-66-8
131	2,2′,3,3′,4,6-Hexachlorobiphenyl	61798-70-7
132	2,2′,3,3′,4,6′-Hexachlorobiphenyl	38380-05-1
133	2,2′,3,3′,5,5′-Hexachlorobiphenyl	35694-04-3
134	2,2′,3,3′,5,6-Hexachlorobiphenyl	52704-70-8
135	2,2′,3,3′,5,6′-Hexachlorobiphenyl	52744-13-5
136	2,2′,3,3′,6,6′-Hexachlorobiphenyl	38411-22-2
137	2,2′,3,4,4′,5-Hexachlorobiphenyl	35694-06-5
138	2,2′,3,4,4′,5′-Hexachlorobiphenyl	35065-28-2
139	2,2′,3,4,4′,6-Hexachlorobiphenyl	56030-56-9
140	2,2′,3,4,4′,6′-Hexachlorobiphenyl	59291-64-4
141	2,2′,3,4,5,5′-Hexachlorobiphenyl	52712-04-6
142	2,2′,3,4,5,6-Hexachlorobiphenyl	41411-61-4
143	2,2′,3,4,5,6′-Hexachlorobiphenyl	68194-15-0
144	2,2′,3,4,5′,6-Hexachlorobiphenyl	68194-14-9
145	2,2′,3,4,6,6′-Hexachlorobiphenyl	74472-40-5
146	2,2′,3,4′,5,5′-Hexachlorobiphenyl	51908-16-8
147	2,2′,3,4′,5,6-Hexachlorobiphenyl	68194-13-8
148	2,2′,3,4′,5,6′-Hexachlorobiphenyl	74472-41-6
149	2,2′,3,4′,5′,6-Hexachlorobiphenyl	38380-04-0
150	2,2′,3,4′,6,6′-Hexachlorobiphenyl	68194-08-1
151	2,2′,3,5,5′,6-Hexachlorobiphenyl	52663-63-5
152	2,2′,3,5,6,6′-Hexachlorobiphenyl	68194-09-2
153	2,2′,4,4′,5,5′-Hexachlorobiphenyl	35065-27-1
154	2,2′,4,4′5,6′-Hexachlorobiphenyl	60145-22-4
155	2,2′,4,4′,6,6′-Hexachlorobiphenyl	33979-03-2
156	2,3,3′,4,4′,5-Hexachlorobiphenyl	38380-08-4
157	2,3,3′,4,4′,5′-Hexachlorobiphenyl	69782-90-7
158	2,3,3′,4,4′,6-Hexachlorobiphenyl	74472-42-7
159	2,3,3′,4,5,5′-Hexachlorobiphenyl	39635-35-3
160	2,3,3′,4,5,6-Hexachlorobiphenyl	41411-62-5
161	2,3,3′,4,5′,6-Hexachlorobiphenyl	74472-43-8
162	2,3,3′,4′,5,5′-Hexachlorobiphenyl	39635-34-2
163	2,3,3′,4′,5,6-Hexachlorobiphenyl	74472-44-9
164	2,3,3′,4′,5′-Hexachlorobiphenyl	74472-45-0

BZ&IUPAC#	IUPAC Name	CASRN
165	2,3,3′,5,5′,6-Hexachlorobiphenyl	74472-46-1
166	2,3,4,4′,5,6-Hexachlorobiphenyl	41411-63-6
167	2,3,4,4′,5,5′-Hexachlorobiphenyl	52663-72-6
168	2,3,4,4′,5′,6-Hexachlorobiphenyl	59291-65-5
169	3,3′,4,4′,5,5′-Hexachlorobiphenyl	32774-16-6
170	2,2′,3,3′,4,4′,5-Hexachlorobiphenyl	35065-30-6
171	2,2′,3,3′,4,4′,6-Hexachlorobiphenyl	52663-71-5
172	2,2′3,3′4,5,5′-Hexachlorobiphenyl	52663-74-8
173	2,2′,3,3′,4,5,6-Hexachlorobiphenyl	68194-16-1
174	2,2′,3,3′,4,5,6′-Hexachlorobiphenyl	38411-25-5
175	2,2′,3,3′,4,5′,6-Hexachlorobiphenyl	40186-70-7
176	2,2′,3,3′,4,6,6′-Hexachlorobiphenyl	52663-65-7
177	2,2′,3,3′,4,5′,6′-Hexachlorobiphenyl	52663-70-4
178	2,2′,3,3′,5,5′,6-Hexachlorobiphenyl	52663-67-9
179	2,2′,3,3′,5,6,6′-Heptachlorobiphenyl	52663-64-6
180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	35065-29-3
181	2,2′,3,4,4′,5,6-Heptachlorobiphenyl	74472-47-2
182	2,2′,3,4,4′,5,6′-Heptachlorobiphenyl	60145-23-5
183	2,2′,3,4,4′,5′,6-Heptachlorobiphenyl	52663-69-1
184	2,2′,3,4,4′,6,6′-Heptachlorobiphenyl	74472-48-3
185	2,2′,3,4,5,5′,6-Heptachlorobiphenyl	52712-05-7
186	2,2′,3,4,5,6,6′-Heptachlorobiphenyl	74472-49-4
187	2,2′,3,4′,5,5′,6′-Heptachlorobiphenyl	52663-68-0
188	2,2′,3,4′,5,6,6′-Heptachlorobiphenyl	74487-85-7
189	2,3,3′,4,4′,5,5′-Heptachlorobiphenyl	39635-31-9
190	2,3,3′,4,4′,5,6-Heptachlorobiphenyl	41411-64-7
191	2,3,3′4,4′,5′,6-Heptachlorobiphenyl	74472-50-7
192	2,3,3′,4,5,5′,6-Heptachlorobiphenyl	74472-51-8
193	2,3,3′,4′,5,5′,6-Heptachlorobiphenyl	69782-91-8
194	2,2′,3,3′,4,4′,5,5′-Octachlorobiphenyl	35694-08-7
195	2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl	52663-78-2
196	2,2′,3,3′,4,4′,5,6′-Octachlorobiphenyl	42740-50-1
197	2,2′,3,3′,4,4′,6,6′-Octachlorobiphenyl	33091-17-7
198	2,2′,3,3′,4,5,5′,6-Octachlorobiphenyl	68194-17-2
199	2,2′,3,3′,4,5,5′,6′-Octachlorobiphenyl	52663-75-9
200	2,2′,3,3′,4,5,6,6′-Octachlorobiphenyl	52663-73-7
201	2,2′,3,3′,4,5′,6,6′-Octachlorobiphenyl	40186-71-8
202	2,2′,3,3′,5,5′,6,6′-Octachlorobiphenyl	2136-99-4
203	2,2′,3,4,4′,5,5′,6-Octachlorobiphenyl	52663-76-0
204	2,2′,3,4,4′,5,6,6′-Octachlorobiphenyl	74472-52-9
205	2,3,3′,4,4′,5,5′,6-Octachlorobiphenyl	74472-53-0
206	2,2′,3,3′,4,4′,5,5′,6-Octachlorobiphenyl	40186-72-9
207	2,2′,3,3′,4,4′,5,6,6′-Octachlorobiphenyl	52663-79-3
208	2,2′,3,3′,4,5,5′,6,6′-Octachlorobiphenyl	52663-77-1
209	Dechachlorobiphenyl	2051-24-3

HOMOLOGS

BZ&IUPAC#	IUPAC Name	CASRN
	Monochlorobiphenyl	27323-18-8
	Dichlorobiphenyl	25512-42-9
	Tichlorobiphenyl	25323-68-6
	Tetrachlorobiphenyl	26914-33-0
	Pentachlorobiphenyl	25429-29-2
	Hexachlorobiphenyl	26601-64-9
	Heptachlorobiphenyl	28655-71-2
	Octachlorobiphenyl	55722-26-4
	Nonachlorobiphenyl	53742-07-7

MIXTURES		
BZ&IUPAC#	**IUPAC Name**	**CASRN**
	Aroclor 1016	12674-11-2
	Aroclor 1210	147601-87-4
	Aroclor 1216	151820-27-8
	Aroclor 1221	11104-28-2
	Aroclor 1231	37234-40-5
	Aroclor 1232	11141-16-5
	Aroclor 1240	71328-89-7
	Aroclor 1242	53469-21-9
	Aroclor 1248	12672-29-6
	Aroclor 1250	165245-51-2
	Aroclor 1252	89577-78-6
	Aroclor 1254	11097-69-1
	Aroclor 1260	11096-82-5
	Aroclor 1262	37324-23-5
	Aroclor 1268	11100-14-4
	Aroclor (unspecified)	12767-79-2

Appendix 3

Carcinogens Listed in the Tenth Report on Carcinogens, 2002

Source: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC.

Bold entries indicate new or changed listing in *The Report on carcinogens, Tenth Edition.*

Part A. Known to be a Human Carcinogen

Name or synonym
Aflatoxins
Alcoholic beverage consumption
4-Aminobiphenyl
Analgesic Mixtures Containing Phenacetin
Analgesic Mixtures Containing
Arsenic Compounds, Inorganic
Asbestos
Azathioprine
Benzene
Benzidine
Beryllium and Beryllium Compounds
1,3-Butadiene
1,4-Butanediol Dimethylsulfonate (Myleran *)
Cadmium and Cadmium Compounds
Chlorambucil
1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU)
bis(Chloroethyl)Ether and Tehnical-Grade chloromethyl Methyl Ether
Chromium Hexavalent Compounds
Coal Tar Pitches
Coal Tars
Coke Oven Emissions
Cyclophosphamide
Cyclosporin A (Ciclosporin)
Diethylstilbestrol
Dyes Metabolized to Benzidine
Environmental Tobacco Smoke
Erionite
Estrogens, Steroidal
Ethylene Oxide
Melphalan
Methoxsalen with Ultraviolet A Therapy (PUVA)
Mineral Oils (Untreated and Mildly Treated)
Mustard Gas
2-Naphthylamine

Name or synonym
Nickel Compounds (See Metallic Nickel and Nickel Compounds)
Radon
Silica, Crystalline (Respirable Size)
Smokeless Tobacco
Solar Radiation
Soots
Strong Inorganic Acid Mists Containing Sulfuric Acid
Sunlamps or Sumbeds, Exposure to (See Ultraviolet Radiation Related Exposures)
Tamoxifen
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), ''Dioxin''
Thiotepa
Thorium Dioxide
Tobacco Smoking
Vinyl Chloride
Ultraviolet Radiation, Broad Spectrum UV Radiation
Wood Dust

Part B. Reasonably Anticipated to be a Human Carcinogen

Name or synonym
Acetaldehyde
2-Acetylaminofluorene
Acrylamide
Acylonitrile
Adriamycin* (Doxorubicin Hydrochloride)
2-Aminoanthraquinone
o-Aminoazotoluene
1-Amino-2-methylanthraquinone
2-Amino-3methylimidazo[4,5-f]quinoline (IQ)
Amitrole
o-Anisidine Hydrochloride
Azacitidine (5-Azacytidine*,5-AzaC)
Benz[a]anthracene(See Polycyclic Aromatic Hydrocarbons)
Benzo[b]fluoranthene(See Polycyclic Aromatic Hydrocarbons)
Benzo[j]fluoranthene(See Polycyclic Aromatic Hydrocarbons)
Benzo[k]fluoranthene(See Polycyclic Aromatic Hydrocarbons)
Benzo[a]pyrene(See Polycyclic Aromatic Hydrocarbons)
Benzotrichloride
Bromodichloromethane
2,2-bis-(Bromoethyl)-1,3-propanediol (Technical Grade)
Butylated Hydroxyanisole (BHA)
Carbon Tetrachloride
Ceramic Fibers (Respirable Size)
Chloramphenicol
Chlorendic Acid
Chlonnated Parans (C12-60% Chlorine)
1-(2-Chloroethy)-3-cyclohexyl-1-nitrosourea
bis(Chloroethyl)nitrosouarea
Chloroform
3-chloro-2-methylpropene
4-chloro-o-phenylenediamine
Chloroprene
p-Chloro-o-toluidine and *p*-Chloro-o-toluidine Hydrochloride (See *p*-Chloro-o-toluidine and *p*-Chloro-o-toluidine Hydrochloride)
Chlorozotocin
C.I.Basic Red 9 Monohydrochloride
Cisplatin

Name or synonym
p-Cresidine
Cupferron
Dacarbazine
Danthron (1,8-Dihydreoxyanthraquinone)
2,4-Diaminoanisole Sulfate
2,4-Diaminotoluene
Dibenz[a,h]acridine (See Polycyclic Aromatic Hydrocarbons)
Dibenz[a,j]acridine (See Polycyclic Aromatic Hydrocarbons)
Dibenz[a,h]anthracene (See Polycyclic Aromatic Hydrocarbons)
7*H*-Dibenzo[c.g]carbazole (See Polycyclic Aromatic Hydrocarbons)
Dibenzo[a,e]pyrene (See Polycyclic Aromatic Hydrocarbons)
Dibenzo[a,h]pyrene (See Polycyclic Aromatic Hydrocarbons)
Dibenzo[a,i]pyrene (See Polycyclic Aromatic Hydrocarbons)
Dibenzo[a,l]pyrene (See Polycyclic Aromatic Hydrocarbons)
1,2-Dibromo-3-chloropropane
1,2-Dibromoethane (Ethylene dibromide)
2,3-Dibromo-1-propanol
tris(s,3-Dibromopropyl)Phosphate
1,4-Didhlorobenzene
3,3′-Dichlorobenzidine and 3,3′-Dichlorobenzidine Dihydrochloride (See 3,3′-Dichlorobenizidine and 3,3′-Dichlorobenzidine Dihydrochloride)
Dichlorodiphenyltrichlorethane (DDT)
1,2-Dichloroethane (Etheylene Dichloride)
Dichloromethane (Methylene Chloride)
1,3-dichloropropene (Technical Grade)
Diepoxybutane
Diesel Exhaust Particulates
Diethyl Sulfate
Diglycidyl Resorcinol Ether
3,3′-dimethoxybenzidine (See 3,3′-Dimethoxybenzidine and Dyes Metabolized to 3,3′-Dimethoxybenzidine)
4-Dimethylaminoazobenzene
3,3′-Dimethylbenzidine (See 3,3′-Dimethoxybenzidine and Dyes Metabolized to 3,3′-Dimethoxybenzidine)
Dimethylcarbamoyl Chloride
1,1-Dimethylhydrazine
Dimethyl Sulfate
Dimethylvinyl Chloride
1,6-Dinitropyrene (See Nitroarenes)
1,8-Dinitropyrene (See Nitroarenes)
1,4-Dioxane
Disperse Blue 1
Dyes Metabolized to 3,3′-Dimethoxybenzidine (See 3,3′-Dimethoxybenzidine and Dyes Metabolized to 3,3′-Dimethoxybenzidine)
Dyes Metabolized to 3,3′-Dimethylbenzidine (See 3,3′-Dimethoxybenzidine and Dyes Metabolized to 3,3′-Dimethoxybenzidine)
Eprichlorohydrin
Ethylene Thiourea
di(2-Ethylhexyl) Phthalate
Ethyl Methanesulfonate
Formaldehyde (Gas)
Furan
Glasswool (Respirable Size)
Glycidol
Hexachlorobenzene
Hexachlorocyclohexane Isomoers
Hexachloroethane

Name or synonym
Hexamethylphosphoramide
Hydrazine and Hydrazine Sulfate (See Hydrazine an Hydrazine Sulfate)
Hydrazobenzene
Indono[1,2,3-cd]pyrene (See Polycyclic Aromatic Hydrocarbons)
Iron Dextran Complex
Isoprene
Kepone*(Chlordecone)
Lead Acetate (See Lead Acetate and Lead Phosphate)
Lead Phosphate (See Lead Acetate and Lead Phosphate)
Lindane and Other Hexachlorocyclohexane Isomers
2-Methylaziridine (Prophlenimine)
5-Methychrysene (See Polycyclic Aromatic Hydrocarbons)
4,4′-Methylenebis (2-chloroaniline)
4-4′-Methylenebis (*N,N*-dimethyl)benzenamine
4,4′-Mehtylenedianiline and 4,4′-Methylenedianiline Dihydrochloride (See 4,4′-Methylenedianiline and its Dihydrochloride Salt)
Methyleugenol
Methyl Methanesulfonate
N-Mehtyl-*N*-nitro-*N*-nitrosoguanidine
Metronidazole
Michler's Ketone [4,4′-(Dimethylamino)benzophenone]
Mirex
Nickel (Metallic) {See Nickel and Nickel Compounds}
Nitrilotriacetic Acid
o-Nitroanisole
6-Nitrochrysene (See Nitroarenes)
Nitrofen (2,4-dichlorophenyl-*p*-nitrophenyl ether)
Nitrogen Mustard Hydrochloride
2-Nitropropane
1-Nitropyrene (See Nitroarenes)
4- Nitropyrene (See Nitroarenes)
N-Nitrosodi-*n*-butylamine
N-Nitrosodinethanolamine
N-Nitrosodiethylamine
N-Nitrosodimethylamine
N-Nitrosodi-n-propylamine
N-Nitroso-N-ethylurea
4-(N-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone
N-Nitroso-N-methylurea
N-Nitrosomethylvinylamine
N-Nitrosomorpholine
N-Nitrosonomicotine
N-Nitrosopiperidine
N-Nitrosopyrrolidine
N-Nitrososarcosine
Norethisterone
Ochratoxin A
4,4′-Oxydianiline
Oxymetholone
Phenacetin (See Phenacetin and Analegsic Mixtures Containing Phenacetin)
Phenazopyridine Hydrochloride
Phenolphthalein
Phenoxybenzamine Hydrochloride
Phenytoin
Polybrominated Biphenyls (PBBs)
Polychlorinated Biphenyls (PCBs)
Polycylclic Aromatic Hydrocarbons (PAHs)

Name or synonym
Procarbazine Hydrochloride
Progesterone
1,3-Propane Sultone
B-Propiolactone
Propylene Oxide
Propylthiouracil
Reserpine
Safrole
Selenium Sulfide
Streptozotocin
Styrene-7,8-oxide
Sulfallate
Tetrachloroethylene (Perchloroethylene)
Tetrafluoroethylene
Tetranitromethane
Thioacetamide
Thiourea
Toluene Diisocyanate
o-Toluidine and *o*-Toluidine Hydrochloride
Toxaphene
Trichloroethylene
2,4,6-Trichlorophenol
1,2,3-Trichlorpropane
Ultraviolet A Radiation
Ultraviolet B Radiation
Ultraviolet C Radiation
Urethane
Vinyl Bromide
4-Vinyl-1-cyclohexene Diepoxide
Vinyl Fluoride

Appendix 4

A Case Study – United Heckathorn Assessment

The case study below is provided as an example of ecological risk assessment (Chapter 17). The material is based on U.S. Environmental Protection Agency's *Guidelines for Ecological Risk Assessment*, published in 1998.

1. SITE HISTORY AND BACKGROUND

The United Heckathorn site has been a major source of DDT in San Francisco Bay since 1947, when a pesticide blending and packaging plant began operations. Although the pesticide blending and packaging operations ended in 1966, DDT accumulation in mussels near the site remains among the highest detected in the California Mussel Watch program. The site is located on the eastern shoreline of the central bay in the city of Richmond, Richmond Inner Harbor, and other areas. Sediments in the harbor, channels, and soil around the facility are contaminated with DDT, dieldrin, and other persistent chlorinated pesticides. An ecological risk assessment was completed for the site in 1994 by EPA's Environmental Research laboratory in Newport, Oregon.

2. PROBLEM FORMULATION AND CONCEPTUAL MODEL

Central San Francisco Bay provides a habitat for many birds, fish, and invertebrates. Aquatic habitats closest to the site include areas of soft bottom with armored shoreline used by anchovy, surfperch, starry flounder, English sole, herring, and other marine fish. Brooks Island lies at the southern end of the inner harbor, and is vegetated and surrounded by mudflats and patches of eelgrass that are used by Pacific herring. The open-water channels near the site also are used by marine birds and harbor seals.

Contaminants of greatest concern include dieldrin and the DDT metabolites, which are both readily adsorbed to sediment particles. The loading of pesticides into vessels adjacent to the site resulted in direct discharge to the channels, where sediments were highly contaminated. The pesticides were also present in surface water and groundwater and were accumulating in biota at the site. DDT is associated with reproductive impacts in fish-eating birds. DDT metabolites and dieldrin also can be directly toxic to fish and invertebrates at

low concentrations. DDT residues in fish have been associated with reproductive problems, such as early life-stage mortality.

Although not explicitly stated as such in the risk assessment, the assessment endpoints evaluated included the following:

- Protection of the benthic community from direct toxic effects.
- Protection of other aquatic species from direct toxic effects.
- Protection of birds from reproductive effects after food chain transfer.
- Protection of fish from reproductive effects.
- Ensuring that concentrations in edible species do not exceed thresholds for human health concerns.

The risk assessment utilized a thorough suite of measurements to evaluate the assessment endpoints. Sediment sampling formed the foundation for the assessment. Sediment grab samples were collected from a total of 20 stations at the site. Samples for chemical analysis, benthic community evaluation, toxicity testing, interstitial-water chemistry, and laboratory bioaccumulation testing all were taken from the same grab. Surface water, fish, crabs, shrimp, and benthic invertebrates from the site also were analyzed for chemical residues.

3. RISK CHARACTERIZATION

A detailed exposure evaluation was conducted using measurements from the site and equilibrium partitioning theory to support food-web modeling and toxicity evaluations. Contaminants associated with three phases of the sediment matrix were examined (particles, freely dissolved, and associated with dissolved organic matter). Chronic and acute ambient water quality criteria values were exceeded in interstitial water at many of the stations. Organisms sampled near the site contained elevated concentrations of DDT metabolites and dieldrin in tissues; for sessile organisms, the concentrations correlated with sediment concentrations. DDT concentrations in shiner surfperch and bay goby were especially elevated and exceeded the U.S. Food and Drug Administration action levels.

Benthic community evaluations indicated that increasing concentrations of DDT in sediment were associated with a reduction in the number of amphipods (especially after excluding one amphipod species that appeared to be more tolerant) and with an altered Infaunal Index. Ten-day sediment-toxicity tests using *Eohaustollus estuarius* indicated that sediments near the site were significantly toxic to amphipods and that there was a gradient of toxicity away from the site. A toxic unit approach was used to evaluate the contribution of various contaminants present in the samples.

Food-web modeling indicated that sediments appeared to be a significant source of contaminants to birds and that birds would be at risk, based on comparisons with effects thresholds in the literature. Fish-eating birds would need to feed exclusively near the site for 2 months each year to exceed risk

standards. Risk was evaluated on a comparative basis between channels, with the channel nearest the site posing the greatest risk.

4. CONCLUSION

The risk assessment report concluded that the greatest risk was due to DDT compounds present in sediment nearest the site. The Lauritzen Channel was identified as a major contamination source, with tidal action transporting contaminated sediment and water away from the area. Organisms near the site were exposed to and accumulating high levels of DDT compounds.

A food-web model was used to evaluate which areas of the site would need to be remediated to reduce risk to birds and fish to acceptable levels. The Lauritzen and Santa Fe Channels, plus some stations at the end of Richmond Inner Harbor, were considered to require remediation based on concentrations found there in fish tissue. To reach protective concentrations in fish and benthic invertebrates, sediment concentrations would need to be between 200 and 500 g/g organic carbon (OC). Sediment concentrations exceeding 300 g total DDT/g OC were toxic to amphipods, and those exceeding 100 g/g OC had a reduced abundance of amphipods. This minimum effects threshold (100 g/g OC) represents a bulk sediment concentration of 1.9 mg/kg total DDT at 1.9% total organic carbon.

The record of decision for the United Heckathorn site was signed on October 26, 1994, requiring the dredging of all soft bay mud from the Lauritzen Channel and Parr Canal, with monitoring to ensure that remediation goals for the site are achieved. The final remediation goals for the site include that the average sediment concentration be below 0.59 mg/kg total DDT, which should be protective for humans and fish-eating birds.

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ENVIRONMENTAL CHEMISTRY AND TOXICOLOGY

ENVIRONMENTAL TOXICOLOGY

Biological and Health Effects of Pollutants

Second Edition

This second edition of ***Environmental Toxicology*** focuses on the biological and health effects of environmental toxicants on living organisms, stressing the relationship between human activities and the environment, and environmental changes in relation to changing patterns of human diseases. The book discusses metabolism, toxicants and their damage process, and environmental, biological, and nutritional factors that may influence toxicity, as well as several natural defense systems including the mechanisms for detoxification — such as endogenous antioxidants and free radical scavenging enzymes — on a cellular level.

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